Universitat de Lleida

Document downloaded from:

<http://hdl.handle.net/10459.1/65708>

The final publication is available at:

<https://doi.org/10.1016/j.foodhyd.2017.04.006>

Copyright

cc-by-nc-nd, (c) Elsevier, 2017

Està subjecte a una llicència de [Reconeixement-NoComercial-SenseObraDerivada 4.0 de Creative Commons](https://creativecommons.org/licenses/by-nc-nd/4.0/)

Accepted Manuscript

Effect of Sodium alginate incorporation procedure on the physicochemical properties of nanoemulsions

M. Artiga-Artigas, A. Acevedo-Fani, O. Martín-Belloso

PII: S0268-005X(17)30285-0
DOI: 10.1016/j.foodhyd.2017.04.006
Reference: FOOHYD 3854
To appear in: *Food Hydrocolloids*
Received Date: 17 February 2017
Revised Date: 29 March 2017
Accepted Date: 04 April 2017

Please cite this article as: M. Artiga-Artigas, A. Acevedo-Fani, O. Martín-Belloso, Effect of Sodium alginate incorporation procedure on the physicochemical properties of nanoemulsions, *Food Hydrocolloids* (2017), doi: 10.1016/j.foodhyd.2017.04.006

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Highlights

- The incorporation procedure of sodium alginate affects nanoemulsions properties.
- The presence of -OH and $-\text{CO}_2^-$ groups varied after microfluidization.
- Microfluidization causes a partial breaking of sodium alginate.
- Sodium alginate chains are able to rearrange after microfluidization.

Effect of Sodium alginate incorporation procedure on the physicochemical properties of nanoemulsions.

Artiga-Artigas, M.; Acevedo-Fani, A.; Martín-Belloso, O*.

Affiliations

Department of Food Technology, University of Lleida – Agrotecnio Center, Av. Alcalde Rovira Roure 191, 25198, Lleida, Spain.

*Information of the corresponding author: Fax +34 973702596; Tel +34 973702593; email: omartin@tecal.udl.cat

Abstract

The aim of this work was to evaluate the effect of sodium alginate incorporation procedure on the physicochemical properties of nanoemulsions formed by microfluidization. Emulsions prepared consisted of corn oil and Tween 20 as dispersed phase and sodium alginate solution as continuous phase. In order to obtain nanoemulsion A (N_A), both phases were microfluidized together. On the other hand, coarse emulsion without sodium alginate was microfluidized and mixed with a microfluidized (MA) or non-microfluidized (N-MA) sodium alginate solution leading to nanoemulsions B(MA) and B(N-MA), respectively. N_A exhibited the smallest particle size (261nm) and monomodal distributions with a polydispersity index of 0.25. The ζ -potential, viscosity and WI of N_A were -37mV, 22.7mPa·s and 57.28, respectively. Spectroscopic, chromatographic and electron microscopic techniques were used to evaluate changes in microfluidized alginate molecules when they were within emulsions. After microfluidization, alginates suffered depolymerization and further rearrangement, changing the disposition of polymer chains around oil droplets and nanoemulsions characteristics. Thus, these results evidence the significant impact of sodium alginate incorporation procedure on the physicochemical properties of nanoemulsions and how it can affect to the stability of the resulting systems which are going to be used as functional ingredients in food matrices.

Keywords

Sodium alginate; microfluidization; nanoemulsion; infrared spectroscopy; corn oil

Introduction

Nanoemulsions are described as colloidal dispersions consisting of oil droplets, with a diameter in the nano-range scale, dispersed into an aqueous phase (Rao & McClements, 2011). Moreover, the presence of a surfactant capable of adsorbing at the oil/water interface of droplets is required to reduce the surface tension and lead to emulsion formation. Oil-in-water nanoemulsions have shown some advantages compared to conventional emulsions. Nanoemulsions present higher physical stability against gravitational processes like flocculation, creaming, sedimentation and coalescence and enhance functionality due to the increase of the surface area of droplets (Guerra-Rosas, Morales-Castro, Ochoa-Martínez, Salvia-Trujillo, & Martín-Belloso, 2016). Moreover, the use of nanoemulsions let diminish the concentration of each component added to the systems minimizing the alterations in the sensorial properties of the food matrix, and also strong light scattering (McClements, 2011). The incorporation of active compounds like certain vitamins, antioxidants or antimicrobials into food matrices represents a challenge due to their low water solubility and high degradability when they are exposed to external factors such as pH, temperature or light (Borrin, Georges, Moraes, & Pinho, 2016). Nanoemulsions appear as systems that could be able to encapsulate, protect and release these compounds effectively (Salvia-Trujillo & McClements, 2015).

On the other hand, the addition of texture modifiers such as biopolymers to the aqueous phase might be useful to improve nanoemulsions stability by reducing droplets movement. Some of these macromolecules, as it is the case of alginates, are capable to interact with surfactant chains disposed around the oil droplets. Hence, they may cause steric and/or electrostatic repulsions between droplet interfaces avoiding destabilization phenomena such as droplets coalescence or gravitational separation (Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2014). Alginates are linear polysaccharides, which consist of β -D-mannuronic (M) acid and α -L-guluronic (G) acid

linked by 1→4 bonds that are able to absorb large quantities of water leading to the formation of hydrogels and hence, facilitating the solubility of the alginate chains in the aqueous phase (Lee & Mooney, 2012; Wang, Sun, Raddatz, & Chen, 2013). In addition, alginates contain functional groups such as carboxylates, which can easily dissociate in the aqueous phase and as a result, provide negative charge to the emulsions (Pereira et al., 2013). Therefore, in presence of multivalent ions, alginates can form polymer networks by a process known as ionic gelation due to their high “gel-forming” capability (Liling et al., 2016). In these networks, linear chains of alginate are connected by ionic interactions resulting in crosslinks called “egg-box”. Taking advantage of this property, nanoemulsions containing alginates could be used as films or edible coatings for food matrices (Strobel, Scher, Nitin, & Jeoh, 2016).

The addition procedure of alginate could cause either the increase of nanoemulsions stability or the destabilization of the blends resulting in the loss of functionality. Moreover, it is thought that polysaccharides suffer modifications after microfluidization process due to high shear stress, impact force and hydrodynamic cavitation, which increase the porosity of the biopolymer molecules extending their superficial area (Laneuville, Turgeon, & Paquin, 2013). In this regard, changes in alginate molecules disposed around oil droplets may alter the physicochemical characteristics of nanoemulsions. Therefore, it is necessary to understand the interactions among the alginate and the rest of the components of the nanostructured systems to obtain nanoemulsions with the most appropriated properties according to their further applications.

Thus, the aim of this work was to assess the effect of the incorporation procedure of alginate on the physicochemical properties of nanoemulsions formed by microfluidization to ensure the stability of the resulting systems during the time. And in turn, what changes may cause microfluidization in sodium alginate structure.

1. Materials and methods

2.1. Materials

Corn oil (Koipesol Asua, Deoleo, Spain) and ultrapure water, obtained from a Millipore Milli-Q filtration system (Merck, Darmstadt, Germany) were used for the preparation of all emulsions. Tween 20 was purchased from Panreac (Barcelona, Spain). Sodium alginate (MANUCOL® DH) was provided by FMC Biopolymers Ltd (Scotland, U.K.). Information provided by the manufacturer indicates that viscosity and pH of a solution 1% is 40-90 mPa·s and 5.0-7.5, respectively.

2.2. Methods

2.2.1. Nanoemulsions formation

All the oil-in-water emulsions showed a final formulation consisting of 1% w/w lipid phase (corn oil) with 1% w/w of surfactant (Tween 20) and 98% w/w aqueous phase (1% w/w sodium alginate, 99% w/w water). Sodium alginate solutions were prepared by dissolving the sodium alginate in water at 70°C for 2 h and then cooling it to room temperature. Concerning nanoemulsions preparation, two different procedures were performed in order to evaluate the effect of the sodium alginate incorporation in the properties of nanoemulsions. After characterizing nanoemulsions in terms of particle size, ζ -potential, viscosity, whiteness index and morphology, those nanoemulsions with the most suitable properties, such as lower particle sizes and ζ -potential values, were more deeply studied to assess the effect of microfluidization in sodium alginate structure.

2.2.1.1. Sodium alginate incorporation before coarse emulsion formation

A coarse emulsion (C_A), was prepared by mixing both lipid and aqueous phases and the surfactant

using a high-speed blender (T25 digital Ultra-Turrax, IKA, Staufen, Germany) at 11.000 rpm for 2 min, at room temperature. C_A was treated with a microfluidizer (M110P, Microfluidics, Massachusetts, USA) at 150 MPa and 5 cycles, in order to obtain nanoemulsion A (N_A). At the outlet of the interaction chamber of the microfluidizer, the product was refrigerated through an external coil immersed in a water bath with ice maintaining the temperature of the samples below 20 °C.

2.2.1.2. Sodium alginate incorporation after coarse emulsion formation

The lipid phase, surfactant and ultrapure water were homogenized with the high-speed blender (T25 digital Ultra-Turrax, IKA, Staufen, Germany) at 11.000 rpm for 2 min, at room temperature. The coarse emulsion (C_B) was passed across the microfluidizer (M110P, Microfluidics, Massachusetts, USA) at 150 MPa for 5 cycles to produce a fine emulsion. This fine emulsion was stirred together with a microfluidized alginate solution (MA) (5 cycles and at 150 MPa) or with a non-microfluidized alginate solution (N-MA), both in the corresponding concentration to obtain a final blend with 1% w/w of sodium alginate, during 1 h at room temperature; this led to nanoemulsion B(MA) ($N_{B(MA)}$) or nanoemulsion B(N-MA) ($N_{B(N-MA)}$), respectively.

2.2.2. Physicochemical characterization

2.2.2.1. Particle size and size distributions

The particle size distribution, polydispersity indexes (Pdl) and mean droplet diameters (nm) of coarse emulsions and nanoemulsions as well as the particle size of alginate solutions were measured using a laser diffractometer (Nano-ZS Zetasizer, Malvern Instruments, Worcestershire, U.K.) working at 633 nm, 25 °C and equipped with a backscatter detector (173°). Samples were prior diluted in ultrapure water using a dilution ratio of 1:9 sample-to-solvent.

2.2.2.2. ζ -potential

The ζ -potential (mV) of oil droplets in emulsions and nanoemulsions was measured by phase-analysis light scattering (PALS) with a Zetasizer NanoZS laser diffractometer (Malvern Instruments Ltd, Worcestershire, UK). Samples were prior diluted in ultrapure water using a dilution ratio of 1:9 sample-to-solvent.

2.2.2.3. Apparent viscosity

Apparent viscosity measurements (mPa·s) of 10 mL aliquots of coarse emulsions, nanoemulsions and alginate solutions were performed by using a vibro-viscometer (SV-10, A&D Company, Tokyo, Japan) vibrating at 30 Hz and constant amplitude (0.4 mm). The device can detect accurate temperature immediately because the fluid and the detection unit (sensor plates) with small surface area/thermal capacity reach the thermal equilibrium in only a few seconds. Therefore, the measurements were performed at controlled room temperature.

2.2.2.4. Whiteness index

The optical properties of coarse emulsions and nanoemulsions were measured using a colorimeter (Konica Minolta CR-400, Konica Minolta Sensing Inc., Osaka, Japan) at room temperature set up for illuminant D65 and 10° observer angle. The device was calibrated with a standard white plate (Y=94.0; x=0.3133; y=0.3194). Once determined the values of CIE L^* , a^* and b^* ; the Whiteness Index (WI) was calculated with equation (1) (Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2013):

$$WI = 100 - ((100 - L)^2 + (a^2 + b^2))^{0.5} \quad \text{eq.(1)}$$

2.2.2.5. Transmission electron microscopy (TEM)

Emulsions were observed by negative-staining electron microscopy as a direct measurement of their droplet size and shape. Conventional emulsions and nanoemulsions were diluted with ultrapure

water using a factor of 1:10, and they were adsorbed onto carbon-coated copper/palladium grids for 1 min. Then, the grids were washed three times by floating it face-down on drops of sterilized, deionized water for 1 min. Finally, the sample was negatively stained by floating the grids face-down on a drop of 2% (w/w) ammonium molybdate at pH 6.5 for 1 min. The grids were observed in a transmission electron microscope (Jeol 2100, FEI Company, Netherlands) at an acceleration voltage of 100 kV.

2.2.3. Molecular characterization

2.2.3.1. Infrared Spectroscopy (ATR)

Infrared spectroscopy through attenuated total reflectance (ATR) was applied to freeze-dried samples of coarse emulsions, nanoemulsions and alginate solutions in order to avoid the interferences caused by water in the –OH signal (3400-3600 cm⁻¹). After freeze-dried process with a Cryodos 50 freeze drier (Telstar Cryodos, Terrassa, Spain) working with a vacuum pressure of 0.128 mBar and a temperature of -45.6 °C, emulsions and alginate solutions were directly measured in solid state by using an infrared spectrometer (FT-IR 6300 Jasco Series, Easton, USA) in a range of 4000-600 cm⁻¹. All tests were performed in triplicate and data were processed using SpectraManager Software (Jasco Analitica Spain S.L, Madrid, Spain).

2.2.3.2. Nuclear magnetic resonance (¹H-NMR)

Samples of emulsions, nanoemulsions and alginate solutions were freeze-dried using a Cryodos 50 freeze drier (Telstar Cryodos, Terrassa, Spain), weighed in a precision scale (Denver Instrument S-234, Germany) and dissolved in 7.5 mL of D₂O in order to be analyzed with a NMR spectrometer (Mercury Plus AS 400 MHz, Varian; California, USA). Data were processed with MestReNova 10.0.2 Software (Mestrelab Research, Santiago de Compostela, Spain).

2.2.3.3. *Gel permeation chromatography (GPC)*

Microfluidized and non-microfluidized alginate solutions were prior diluted in water by a 1:1 factor and compared with different dextran solutions of 2 mg/mL acting as patterns (Sigma-Aldrich, USA).

Gel permeation chromatography (GPC) of the alginate solutions was used to study the breaking of alginate molecules. The chromatograph (Waters Alliance 2695, Waters, Barcelona, Spain) was composed by a refraction index detector (Waters 2414, Barcelona, Spain) and a weighing scale (Mettler Toledo AT261 Delta Range, Barcelona, Spain). Chromatographic conditions consisted of an Ultrahydrogel 1000 + Ultrahydrogel 250, 300x7.8 mm column (Waters, Barcelona, Spain) using NaNO_3 0.1M as eluent at 30°C and the injection volume was 50 μl with a flux of 1.0 mL/min. Data were processed with the Empower GPC Software (Waters, Mildford, USA).

2.3. *Statistics*

All the procedures were assessed in duplicate, and at least three measurements of each parameter were carried out. The statistical software SigmaPlot 11.0 (Systat Software Inc., Pennsylvania, USA) was used to perform the analysis of variance. To determine differences among means of the different procedures, Tukey test (Two Way ANOVA) was run at a 5% significance level. In the case of comparing microfluidized and non-microfluidized alginate solutions, a Rank Sum test (One Way ANOVA) was run at a 5% significance level as well.

3. *Results*

3.1. *Average droplet size*

Average particle sizes of emulsions and nanoemulsions obtained by the different procedures are shown in Table 1. All coarse emulsions exhibited a dramatic reduction of their average droplet size after creating shear and impact forces caused by microfluidization (Maa & Hsu, 1999). $N_{B(MA)}$ and

$N_{B(N-MA)}$, in which alginate solution (microfluidized or not, respectively) was added in the last step of the nanoemulsion preparation, presented average droplet sizes over 300 nm with values that did not show statistically significant differences ($P>0.05$).

3.2. Polydispersity indexes and droplet size distributions

Polydispersity indexes (PdI) of N_A decreased after microfluidization regarding their respective coarse emulsions (Table 1). In the case of $N_{B(MA)}$ and $N_{B(N-MA)}$, PdI values increased after microfluidization without significant differences ($P<0.05$) neither between the coarse emulsions nor between the nanoemulsions. Moreover, as it can be appreciated in Figure 1, $N_{B(MA)}$ and $N_{B(N-MA)}$ exhibited multimodal droplet size distributions with major intensity peaks positioned at the nano-range scale and with minor peaks not only located at the macro-range but above the detection limit of the equipment (more than 10,000 nm), even after microfluidization process of sodium alginate as it is the case of $N_{B(MA)}$. Multimodal distributions led to high polydispersity indexes (PdI), hence, N_A showed the lowest PdI with a value of 0.25 ± 0.01 , while the PdI of $N_{B(MA)}$ and $N_{B(N-MA)}$ did not present significant differences with values between 0.41 ± 0.02 and 0.45 ± 0.02 , respectively.

3.3. ζ -potential

The ζ -potential values of emulsions and their respective nanoemulsions obtained were all negative as it can be observed in Table 1. Moreover, the negative electrical charge of droplets in N_A and $N_{B(MA)}$ decreased after microfluidization regarding their respective coarse emulsions. However, the ζ -potential of $N_{B(N-MA)}$ did not change significantly ($P<0.05$) concerning their coarse emulsion.

3.4. Apparent Viscosity

C_B , without alginate, presented rather low viscosity values and similar to the water viscosity (≈ 1 mPa·s), whereas C_A exhibited viscosity values of 42.4 ± 0.8 mPa·s on average (Table 1). The highest increase after microfluidization was observed for $N_{B(N-MA)}$, where the non-microfluidized

alginate solution was mixed with the fine emulsion observing a viscosity value of 61.07 mPa·s on average. However, in the case of using microfluidized alginate ($N_{B(MA)}$) the viscosity was lower (Table 1).

Oppositely, the viscosity of N_A and alginate solutions (Fig. 2B) decreased on average from 42.43 to 22.73 mPa·s and from 167 to 137 mPa·s, respectively, after microfluidization process.

3.5. Whiteness index (WI)

The optical properties of coarse emulsions and nanoemulsions expressed as WI are presented in Table 1. WI values increased after microfluidization process in all cases. Nevertheless, WI of N_A and $N_{B(MA)}$, in which alginate solution underwent to the microfluidization process, did not differ significantly. Oppositely, $N_{B(N-MA)}$ showed the highest WI with a value of 60.28 ± 0.05 .

3.6. Microstructure

The morphology of droplets in N_A , $N_{B(MA)}$ and $N_{B(N-MA)}$ was examined by transmission electron microscopy (TEM) as it can be observed in Figure 3. With this technique, it was possible to observe significant differences regarding the shape and the disposition of the droplets. TEM images revealed a wide range of particle sizes with an average droplet size of 183 ± 45 nm in N_A (Fig.3A), whereas its mean particle size calculated by DLS was higher (261 ± 6 nm). In the case of $N_{B(MA)}$ where alginate solution was microfluidized and added in the last step of emulsion formation, TEM images presented discrete and spherical particles whose droplet diameters ranged between 10 to 500 nm (Fig.3B).

Nevertheless, as in the case of DLS, in TEM images of $N_{B(N-MA)}$ (Fig.3C) it is possible to confirm that the presence of non-microfluidized sodium alginate led to particles with the highest mean droplet diameters 382 ± 209 nm and there were not isolated particles (Table 1).

3.7. *Effect of microfluidization on alginate molecular structure.*

N_A presented the lowest droplets sizes, ζ -potential, viscosity and WI values, thus, they were expected to be the nanoemulsions with the most suitable properties to be stable during the time. In order to study the effects of microfluidization process on polymer structure, chemical and structural characterizations of coarse emulsions and nanoemulsions A (C_A and N_A , respectively) and alginate solutions by FT-IR and 1H -NMR spectroscopies and gel permeation chromatography (GPC), were achieved.

3.7.1. *FT-IR and 1H -NMR spectra*

Microfluidized alginate spectrum showed a slight decrease of the $-OH$ (3300 cm^{-1} , stretch, H-bonded) and $-COO^-$ groups (1593 and 1400 cm^{-1} , asymmetric and symmetric stretch, respectively) after microfluidization, being the signal corresponding to $-OH$ groups who experienced the highest decrease (Fig.4A).

Regarding emulsions spectra, Figure 4B showed the disappearance of the peaks at 3002 and 1633 cm^{-1} (alkenes, $-C=C-$) and 1740 cm^{-1} (together with 1100 cm^{-1} , ester, $R'COO-R$) that correspond exclusively to corn oil. This could indicate that the corn oil remained within the biopolymer structure. Moreover, the band corresponding to the esters of Tween 20 (1731 cm^{-1} , $R'COO-R$) decreased in intensity after microfluidization process, whereas those peaks corresponding to alkenes (2919 and 1624 cm^{-1}) did not experiment significant variations ($P>0.05$) in emulsions spectrum (Fig.4B). Nevertheless, it was possible to observe a slight increase in the signal corresponding to O-H bond (3330 cm^{-1} , stretch, H-bonded) and carboxylate groups ($-C=OO^-$, 1595 and 1402 cm^{-1} , asymmetric and symmetric stretch, respectively) of sodium alginate in nanoemulsion spectrum in comparison to the coarse emulsion.

According to proton nuclear magnetic resonance (1H -NMR) technique, microfluidized alginate lost a 31% of the protons in comparison with non-microfluidized alginate spectrum (Fig.5). Likewise,

the number of total protons in the spectrum of the coarse emulsion is approximately a 38% higher than that observed in nanoemulsions (Fig.6A and 6B, respectively), being remarkable that differences in peaks integration occurred only in the region corresponding to the main chain of sodium alginate (3.70 - 4.20 ppm), whereas the rest of integrations remained unaltered (Huang et al., 2015).

3.7.2. Size exclusion chromatography: Gel permeation chromatography (GPC)

Table 2 shows GPC analyses of microfluidized and non-microfluidized alginate solutions. GPC analyses revealed a significant reduction of average molecular weight of microfluidized alginate from $785,656 \pm 48,5461$ u to $679,809 \pm 18,455$ u, together with the change in the shape of the GPC profiles (not reported), which indicated the presence of a second polymer distribution after having passed through the microfluidizer. However, the difference in the average values for the rest of studied variables between the two groups was not great enough to exclude the possibility that the difference was due to random sampling variability and therefore, there was not a statistically significant difference ($P= 0.333$).

4. Discussion

Nanoemulsions A (N_A) in which sodium alginate solution was microfluidized at the same time that the rest of components, showed the smallest particle sizes. During microfluidization, creating shear and impact forces may cause a cavitation phenomenon that leads to the reduction of the emulsions droplet size (Maa & Hsu, 1999). In this sense, alginate chains disposition around the droplets may be more homogeneous because the high shear stress occurred on all components of the nanoemulsion at the same time, resulting in a lower PdI (Qian & McClements, 2011). However, in $N_{B(MA)}$ and $N_{B(N-MA)}$, in which the added alginate was microfluidized or not, respectively; a destabilization phenomenon such as re-coalescence of oil droplets may occur after microfluidization causing an increase in the polydispersity indexes of nanoemulsions (Bonilla, Atarés, Vargas, &

308 **Chiralt, 2012).** Nevertheless, microfluidization had an influence on particle sizes promoting the
309 changes of the molecular structure of alginate, which allow obtaining smaller particle sizes.
310 Consequently, the highest particle sizes were observed for $N_{B(N-MA)}$ in which alginate did not pass
311 through the microfluidizer. Indeed, $N_{B(MA)}$ and $N_{B(N-MA)}$ size distributions were not monomodals
312 (Fig.1) but they presented another minor peak. These minor peaks observed in Fig.1 could be
313 surfactant micelles that were not adsorbed at the oil droplets interface due to an excess of alginate
314 molecules that had been repelled (Heydenreich, Westmeier, Pedersen, Poulsen, & Kristensen, 2003;
315 Neumann, Schmitt, & Iamazaki, 2003). However, it is more likely that they were aggregates of the
316 biopolymer molecules that do not contain oil within, since particle size distributions of both
317 microfluidized and non-microfluidized alginate solutions (Fig.2A) revealed the same minor peak at
318 the same position.

319
320 Moreover, particle size and polydispersity indexes variability between N_A , $N_{B(MA)}$ and $N_{B(N-MA)}$
321 observed using light scattering was confirmed by TEM (Fig.3). Certainly, in these images it was
322 possible to observe that the presence of aggregates is higher if alginate molecules are not subjected
323 to high shear stress (Fig.3C). The lack of microfluidization process might cause that alginate
324 molecules maintain their original structure without suffering depolymerization processes, which
325 may cause greater steric impediment among biopolymer chains during their disposition around the
326 oil droplets leading to bigger and non-spherical particles. Thus, microfluidization may also enhance
327 the stability of the blends (Feng, Cao, Xu, You, & Han, 2016; Villay et al., 2012).

328
329 On the other hand, Tween 20 is a non-ionic low-mass surfactant that rapidly coats the newly created
330 droplets during emulsification (Kralova & Sjöblom, 2009) giving a neutral or slightly negative
331 electrical charge on oil-water interface (Hsu & Nacu, 2003). Nevertheless, a negative charge was
332 observed in all the emulsions and nanoemulsions in the present work. This is because Tween 20 is
333 preferably adsorbed on the oil surface in the co-presence of polymeric stabilizers, such as sodium

alginate molecules, which provided negative charge to oil-water interfaces when dispersed in the aqueous phase (Nambam & Philip, 2012). Therefore, the alginate addition had a really strong influence in the surface charge of oil droplets in emulsions and nanoemulsions due to the presence of carboxylates and hydroxyl groups, which are easily deprotonated at neutral pH ($\approx 6-7$) (J. S. Yang, Jiang, He, & Xia, 2012). Furthermore, the mechanical stress during microfluidization can lead alginate chains to break up or modify its disposition, increasing the number of those molecules able to be potentially adsorbed on the oil-water interface (Chen, Gao, Yang, & Gao, 2013). In this sense, a partial breaking of alginate molecule may cause the change of its disposition around the oil droplets and increase the concentration of deprotonated groups. In this sense, N_A showed the lowest ζ -potential value and therefore, it could be expected that this nanoemulsion will be less exposed to re-coalescence than $N_{B(MA)}$ and $N_{B(N-MA)}$.

The increase in viscosity values of the obtained emulsions and nanoemulsions depended on the fact that the alginate has been microfluidized or not. According to Yang et al. (2012), apparent viscosity of emulsions is strongly influenced by the presence of the alginate dispersed in the aqueous phase. In fact, alginate may be a good candidate for chemical functionalization because can present surface activity after being dispersed into the aqueous medium, increasing the viscosity of the solutions. In contrast and based on our results, microfluidization process could cause a significant increase in the porosity of polysaccharide chains due to cavitation forces, which allow chains holding more water leading to the decrease of apparent viscosity (Wang et al., 2013). Harte & Venegas, (2010) also reported a 75-85% viscosity reduction in alginate solutions processed by high-pressure homogenization from 1 to 5 cycles up to 300 MPa. Similarly, Villay et al., (2012) observed a viscosity reduction on sodium alginate solutions when they were microfluidized at 195 MPa for several cycles. They attributed this effect to a decrease in the molar mass of sodium alginate due to a depolymerization of chains caused by covalent bond disruption. This depolymerization is a result of the chemical reaction between the polymers and high energy molecules such as hydroxyl radicals

produced from cavitation (Feng, Cao, Xu, Wang, & Zhang, 2017). Only when the molecular weight of sodium alginate (M_w) is low enough, particles are able to resist mechanical forces such as shear, turbulence or cavitation, caused by microfluidization. This fact let diminish the heterogeneity of chains, and thus the PDI of this macromolecular species in solution (Villay et al., 2012).

Although all the coarse emulsions exhibited a decrease in their average droplet size and size distribution after being microfluidized, none of the nanoemulsions of this study became completely transparent. It is reported that large particles scatter the light more intensely and it produces an increase in lightness (L^*), opacity and WI of emulsions (McClements, 2011). Therefore, alginate molecules may contribute to raise WI values, probably due to an increase of the number of large particles in dispersion (McClements, 2002).

In addition, according to Salvia-Trujillo et al. (2014), the type of oil used in the formulation significantly influenced the WI of the coarse emulsions and nanoemulsions. Corn oil, as other vegetal oils, is intense in color and causes an increase of the WI after microfluidization process (Ekthamasut & Akesowan, 2010; Pereda, Aranguren, & Marcovichs, 2010). It is described that the light scattering of oil droplets depends on the refractive index of continuous and dispersed phase, oil concentration and droplet size (McClements, 2002a). In this regard, when the refractive index ratio (n_2/n_1) tends to unity, as in the case of corn oil whose refractive index is 1.47 ($n_1 = 1.33$), the light scattering produced by the lipid droplets dispersed in the emulsion promotes the increase of the opacity of the blends (McClements, 2002a; L. Yang & Paulson, 2000).

The increase of hydroxyls observed in alginates spectrum (Fig. 4B) may be due to the breaking of O-glycosidic bonds (depolymerization) or an opening of the alginate structure caused by mechanical forces into the microfluidizer, which increase the ability of sodium alginate for holding water (Chen et al., 2013; Mohammad, Hosseini, Emam-djomeh, Hadi, & Meeren, 2013). Nonetheless, in spite of microfluidization may accelerate the collisions between the solvent

386 molecules and sodium alginate molecules causing biopolymer degradation, it has been suggested
387 that alginate is able to rearrange (Chemat & Khan, 2011).

388 In contrast, in the case of nanoemulsion spectrum, the observed behavior was the opposite because
389 inside these systems mechanical forces can attack the structure of microfluidized molecules, which
390 were not able to restructure (Laneuville et al., 2013). Indeed, the application of these forces may
391 result in some reactions such as sodium alginate conjugation or oxidation (Feng et al., 2017). In this
392 regard, high shear stress caused by microfluidization process could cause the oxidation of hydroxyl
393 groups to carboxylic acids easily deprotonated in aqueous media, able to increase the carboxylate
394 band of nanoemulsion spectrum. This may fit with the hypothesis of a partial breaking of sodium
395 alginate molecule able to react with high energy molecules such as hydroxyl radicals ($\text{-OH}^\bullet$)
396 produced from cavitation forces into the microfluidizer. This would entail the increase in the
397 amount of exchangeable protons from carboxylic acids or hydroxyl groups (Feng et al., 2017).

398 As a result, those exchangeable protons, which were not observable by ^1H -RMN due to the use of
399 deuterium dioxide as the solvent; led to the decrease in the number of relative protons in
400 microfluidized alginate and nanoemulsion spectra. Thus, results from both spectroscopic techniques
401 indicated that after microfluidization, sodium alginate might break and change its conformation
402 influencing the properties of the resultant nanoemulsions.

403
404 Concerning the results, it could be expected the observation of the breaking of sodium alginate in
405 smaller oligomers through ultra-hydrogel permeation chromatography (GPC). In this regard,
406 although sodium alginate molecule breaks, the re-aggregates formed after microfluidization process
407 may have similar molecular weights than the initial chain.

408 Those changes might be explained by a partial hydrolysis of alginate molecule that causes higher
409 affinity for water, higher porosity and hence, changes in its molecular weight, which might produce
410 a variation in the disposition of alginate chains around the oil droplets.

5. Conclusions

The present work reveals that physicochemical properties of nanoemulsions can vary depending on their preparation procedure. Mechanical forces from microfluidization caused the breaking of alginate chains reducing the steric impediment between them around the oil droplets. Thus, in terms of particle sizes measured by DLS and TEM, those procedures in which the alginate was microfluidized, either before or after coarse emulsion formation, led to nanoemulsions (N_A and $N_{B(MA)}$) with particle sizes in the nano-scale range. On the other hand, results derived from spectroscopic techniques such as ATR and 1H -NMR also fitted with a partial breaking of sodium alginate molecule after passing through the microfluidizer. Consequently, it was possible to observe that the presence of $-OH$ and $-COO^-$ groups varied changing the interfacial interactions between oil droplets and the rest of the components in the resultant nanoemulsions. Moreover, the GPC analysis highlighted the possibility of rearrangement of the biopolymer after microfluidization since, although the molecular weight of the sodium alginate decreased, a molecular weight distribution similar to the non-microfluidized alginate was obtained. Therefore, in order to obtain long term stable nanoemulsions able to encapsulate, transport and release biocompounds more effectively through food matrices, further studies such as Cryo electron microscopy may result interesting to understand the real disposition of the alginate chains around oil droplets.

Acknowledgements

This study was supported by the MINECO (Spain) throughout project AGL2012-35635 and by the MINECO/FEDER throughout project AGL2015-65975-R. Authors María Artiga-Artigas and Alejandra Acevedo-Fani thank the University of Lleida for the pre-doctoral grant.

References

Bonilla, J., Atarés, L., Vargas, M., & Chiralt, A. (2012). Effect of essential oils and homogenization conditions on properties of chitosan-based films. *Food Hydrocolloids*, 26(1), 9–16.

- 438 Borrin, T. R., Georges, E. L., Moraes, I. C. F., & Pinho, S. C. (2016). Curcumin-loaded
439 nanoemulsions produced by the emulsion inversion point (EIP) method: An evaluation of
440 process parameters and physico-chemical stability. *Journal of Food Engineering*, 169, 1–9.
- 441 Chemat, F., & Khan, M. K. (2011). Ultrasonics Sonochemistry Applications of ultrasound in food
442 technology : Processing , preservation and extraction. *Ultrasonics - Sonochemistry*, 18(4),
443 813–835.
- 444 Chen, J., Gao, D., Yang, L., & Gao, Y. (2013). Effect of micro fluidization process on the
445 functional properties of insoluble dietary fiber, 54, 1821–1827.
- 446 Ekthamasut, K., & Akesowan, A. (2010). Effect of Vegetable Oils on Physical Characteristics of
447 Edible Konjac Films. *Water*, (4).
- 448 Feng, L., Cao, Y., Xu, D., Wang, S., & Zhang, J. (2017). Ultrasonics Sonochemistry Molecular
449 weight distribution , rheological property and structural changes of sodium alginate induced by
450 ultrasound. *Ultrasonics - Sonochemistry*, 34, 609–615.
- 451 Feng, L., Cao, Y., Xu, D., You, S., & Han, F. (2016). Ultrasonics Sonochemistry Influence of
452 sodium alginate pretreated by ultrasound on papain properties : Activity , structure ,
453 conformation and molecular weight and distribution. *ULTRASONICS SONOCHEMISTRY*, 32,
454 224–230.
- 455 Guerra-Rosas, M. I., Morales-Castro, J., Ochoa-Martínez, L. A., Salvia-Trujillo, L., & Martín-
456 Belloso, O. (2016). Long-term stability of food-grade nanoemulsions from high methoxyl
457 pectin containing essential oils. *Food Hydrocolloids*, 52, 438–446.
- 458 Harte, F., & Venegas, R. (2010). A Model for Viscosity Reduction in Polysaccharides Subjected To
459 High-Pressure Homogenization. *Journal of Texture Studies*, 41(1), 49–61.
- 460 Heydenreich, a. V., Westmeier, R., Pedersen, N., Poulsen, H. S., & Kristensen, H. G. (2003).
461 Preparation and purification of cationic solid lipid nanospheres - Effects on particle size,

- 462 physical stability and cell toxicity. *International Journal of Pharmaceutics*, 254(1), 83–87.
- 463 Hsu, J.-P., & Nacu, A. (2003). Behavior of soybean oil-in-water emulsion stabilized by nonionic
464 surfactant. *Journal of Colloid and Interface Science*, 259(2), 374–381.
- 465 Huang, J., Li, J., Feng, Y., Li, K., Yan, H., & Gao, P. (2015). Colloids and Surfaces A :
466 Physicochemical and Engineering Aspects Aggregation behavior of derivatives of sodium
467 alginate and N-octyl- β -D -glucopyranoside in aqueous solutions, 479, 11–17.
- 468 Kralova, I., & Sjöblom, J. (2009). Surfactants Used in Food Industry: A Review. *Journal of*
469 *Dispersion Science and Technology*, 30(9), 1363–1383.
- 470 Laneuville, S. I., Turgeon, S. L., & Paquin, P. (2013). Changes in the physical properties of xanthan
471 gum induced by a dynamic high-pressure treatment. *Carbohydrate Polymers*, 92(2), 2327–
472 2336.
- 473 Lee, K. Y., & Mooney, D. J. (2012). Alginate: Properties and biomedical applications. *Progress in*
474 *Polymer Science (Oxford)*, 37(1), 106–126.
- 475 Liling, G., Di, Z., Jiachao, X., Xin, G., Xiaoting, F., & Qing, Z. (2016). Effects of ionic crosslinking
476 on physical and mechanical properties of alginate mulching films. *Carbohydrate Polymers*,
477 136, 259–265.
- 478 Maa, Y. F., & Hsu, C. C. (1999). Performance of sonication and microfluidization for liquid-liquid
479 emulsification. *Pharmaceutical Development and Technology*, 4(2), 233–240.
- 480 McClements, D. J. (2002a). Colloidal basis of emulsion color. *Current Opinion in Colloid &*
481 *Interface Science*, 7(5–6), 451–455.
- 482 McClements, D. J. (2002b). Colloidal basis of emulsion color. *Current Opinion in Colloid and*
483 *Interface Science*, 7(5–6), 451–455.
- 484 McClements, D. J. (2011). Edible nanoemulsions: fabrication, properties, and functional

- performance. *Soft Matter*, 7(6), 2297–2316.
- Mohammad, S., Hosseini, H., Emam-djomeh, Z., Hadi, S., & Meeren, P. Van Der. (2013). Food Hydrocolloids b -Lactoglobulin e sodium alginate interaction as affected by polysaccharide depolymerization using high intensity ultrasound. *FOOHYD*, 32(2), 235–244.
- Nambam, J. S., & Philip, J. (2012). Competitive adsorption of polymer and surfactant at a liquid droplet interface and its effect on flocculation of emulsion. *Journal of Colloid and Interface Science*, 366(1), 88–95.
- Neumann, M. G., Schmitt, C. C., & Iamazaki, E. T. (2003). A fluorescence study of the interactions between sodium alginate and surfactants. *Carbohydrate Research*, 338(10), 1109–1113.
- Pereda, M., Aranguren, M. I., & Marcovich s, N. E. (2010). Caseinate films modified with tung oil. *Food Hydrocolloids*, 24(8), 800–808.
- Pereira, R., Carvalho, A., Vaz, D. C., Gil, M. H., Mendes, A., & Bártolo, P. (2013). Development of novel alginate based hydrogel films for wound healing applications. *International Journal of Biological Macromolecules*, 52, 221–230.
- Qian, C., & McClements, D. J. (2011). Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. *Food Hydrocolloids*, 25(5), 1000–1008.
- Rao, J., & McClements, D. J. (2011). Food-grade microemulsions, nanoemulsions and emulsions: Fabrication from sucrose monopalmitate & lemon oil. *Food Hydrocolloids*, 25(6), 1413–1423.
- Salvia-Trujillo, L., & McClements, D. J. (2015). Influence of Nanoemulsion Addition on the Stability of Conventional Emulsions. *Food Biophysics*.
- Salvia-Trujillo, L., Rojas-Graü, A., Soliva-Fortuny, R., & Martín-Belloso, O. (2014). Food Hydrocolloids Physicochemical characterization and antimicrobial activity of food- grade emulsions and nanoemulsions incorporating essential oils. *Food Hydrocolloids*, 43, 1–10.

- 509 Salvia-Trujillo, L., Rojas-Graü, M. A., Soliva-Fortuny, R., & Martín-Belloso, O. (2013). Effect of
 510 processing parameters on physicochemical characteristics of microfluidized lemongrass
 511 essential oil-alginate nanoemulsions. *Food Hydrocolloids*, 30(1), 401–407.
- 512 Salvia-Trujillo, L., Rojas-Graü, M. A., Soliva-Fortuny, R., & Martín-Belloso, O. (2014).
 513 Formulation of Antimicrobial Edible Nanoemulsions with Pseudo-Ternary Phase Experimental
 514 Design. *Food and Bioprocess Technology*, 3022–3032.
- 515 Strobel, S. A., Scher, H. B., Nitin, N., & Jeoh, T. (2016). In situ cross-linking of alginate during
 516 spray-drying to microencapsulate lipids in powder. *Food Hydrocolloids*, 58, 141–149.
- 517 Villay, a., Lakkis de Filippis, F., Picton, L., Le Cerf, D., Vial, C., & Michaud, P. (2012).
 518 Comparison of polysaccharide degradations by dynamic high-pressure homogenization. *Food*
 519 *Hydrocolloids*, 27(2), 278–286.
- 520 Wang, T., Sun, X., Raddatz, J., & Chen, G. (2013). Effects of microfluidization on microstructure
 521 and physicochemical properties of corn bran. *Journal of Cereal Science*, 58(2), 355–361.
- 522 Yang, J. S., Jiang, B., He, W., & Xia, Y. M. (2012). Hydrophobically modified alginate for
 523 emulsion of oil in water. *Carbohydrate Polymers*, 87(2), 1503–1506.
- 524 Yang, L., & Paulson, A. T. (2000). Effects of lipids on mechanical and moisture barrier properties
 525 of edible gellan film. *Food Research International*, 33(7), 571–578.
- 526

Figure 1. Droplet size distribution in terms of intensity (%) of nanoemulsions A (N_A) where the incorporation of sodium alginate took place before the coarse emulsion formation; and nanoemulsions B where the incorporation of microfluidized ($N_{B(N-MA)}$) or non-microfluidized ($N_{B(MA)}$) sodium alginate, respectively, occurred after coarse emulsion formation. Microfluidization processes were performed at 150 MPa and for 5 cycles.

Figure 2. (A) Average particle size distributions (nm) and (B) apparent viscosity mean values (mPa·s) of non-microfluidized (N-MA) and microfluidized (MA) alginate solutions (at 150 MPa and 5 cycles, respectively).

Figure 3. Transmission electron microscopy (TEM) images of nanoemulsions A (N_A) where the incorporation of sodium alginate took place before the coarse emulsion formation (A); and nanoemulsions B where the incorporation of microfluidized ($N_{B(N-MA)}$) or non-microfluidized ($N_{B(MA)}$) sodium alginate occurred after coarse emulsion formation (B or C, respectively). Microfluidization processes were performed at 150 MPa and for 5 cycles.

Figure 4 Normalized infrared spectra of (A) freeze-dried non-microfluidized (N-MA) and microfluidized (MA) alginate solutions at 150 MPa and 5 cycles and (B) freeze-dried nanoemulsions A (NA) and coarse emulsions A (CA) containing sodium alginate. Microfluidization was performed at 150 MPa, for 5 cycles.

Figure 5 ^1H -NMR comparative spectra of microfluidized (MA) and Non-microfluidized (N-MA) alginate solutions in D_2O at room temperature.

Figure 6 (A) ^1H -NMR spectrum of coarse emulsion A (C_A) and (B) ^1H -NMR spectrum of nanoemulsion A (N_A) in D_2O at room temperature.

Microfluidization:
5 cycles, 150 MPa

Table 1. Droplet size (nm), polydispersity index (PDI), ζ -potential (mV), viscosity (mPa·s) and whiteness index (WI) of coarse emulsions A (C_A) and B (C_B)-with and without containing sodium alginate, respectively- and nanoemulsions N_A where sodium alginate was added in the first step of emulsions formation; $N_{B(MA)}$ and $N_{B(N-MA)}$ in which a solution of microfluidized or non-microfluidized sodium alginate solution, respectively, was added to a fine emulsion prior prepared. Data shown are the means $\pm$ standard deviation.

	<i>Droplet size (nm)</i>	<i>PdI</i>	<i>ζ-potential (mV)</i>	<i>Viscosity (mPa·s)</i>	<i>Color (WI)</i>
C_A	8865 $\pm$ 588 ^a	0.45 $\pm$ 0.14 ^a	-10.3 $\pm$ 0.8 ^a	42.4 $\pm$ 0.8 ^a	52.84 $\pm$ 0.04 ^a
C_B	4012 $\pm$ 555 ^b	0.27 $\pm$ 0.14 ^b	-15.8 $\pm$ 2.1 ^c	1.17 $\pm$ 0.02 ^b	42.65 $\pm$ 1.87 ^c
N_A	261 $\pm$ 6 ^c	0.25 $\pm$ 0.01 ^c	-39 $\pm$ 3 ^d	22.7 $\pm$ 0.4 ^c	57.28 $\pm$ 0.02 ^d
$N_{B(MA)}$	315 $\pm$ 5 ^d	0.41 $\pm$ 0.02 ^d	-20.9 $\pm$ 1.5 ^f	29.5 $\pm$ 0.4 ^e	58 $\pm$ 2 ^d
$N_{B(N-MA)}$	443 $\pm$ 28 ^d	0.45 $\pm$ 0.02 ^d	-26.98 $\pm$ 0.01 ^e	61.07 $\pm$ 0.21 ^d	60.28 $\pm$ 0.05 ^e

^{a,b,c,d,e,f} Means in same column with different letters are significantly different at $p < 0.05$.

Table 2. Number average molecular weight (Mn), weight average molecular weight (Mw), molecular weight of the highest peak (MP), higher average molecular weights (Mz, Mz+1) and polydispersity index (Mw/Mn) obtained by gel permeation chromatography (GPC) of microfluidized (MA) or non-microfluidized (N-MA) alginate solutions. Data shown are the means $\pm$ standard deviation.

	Mn	Mw	MP	Mz	Mz+1	Mw/Mn
MA	95,696 $\pm$ 1,334 ^a	679,809 $\pm$ 18,455 ^b	581,150 $\pm$ 13,799 ^a	1,768,951 $\pm$ 64,090 ^a	2,867,030 $\pm$ 123,798 ^a	7.1 $\pm$ 0.1 ^a
N-MA	98,070 $\pm$ 3,186 ^a	785,656 $\pm$ 48,5461 ^a	606,008 $\pm$ 7,200 ^a	2,265,000 $\pm$ 125,500 ^a	3,874,397 $\pm$ 185,396 ^a	8.17 $\pm$ 0.23 ^a

^{a,b} Means in same column with different letters are significantly different at $p < 0.05$. Data shown are the means $\pm$ standard deviation.