

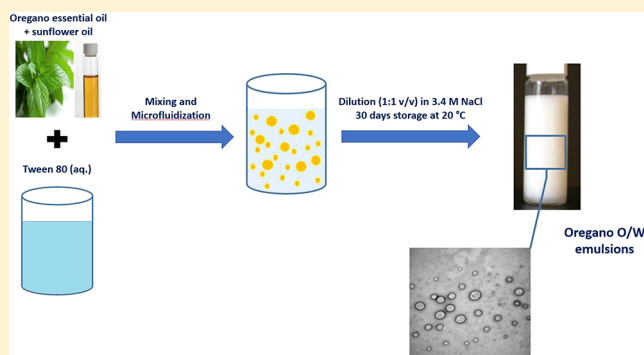
Fundamental Study on the Salt Tolerance of Oregano Essential Oil-in-Water Nanoemulsions Containing Tween 80

Ali Sedaghat Doost,^{*,†} Christian V. Stevens,[‡] Myriam Claeys,[§] and Paul Van Der Meeren[†]

[†]Particle and Interfacial Technology Group (PaInT) and [‡]SynBioC Research Group, Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Gent, Belgium

[§]Nematology Research Unit, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Gent, Belgium

ABSTRACT: This study provides fundamental information about the influence of salt on the physicochemical stability of oregano essential oil (EO) and its main components incorporated in a nanoemulsion delivery system containing Tween 80 (T80) emulsifier. The emulsion stability was found to be strongly correlated with the lipid phase composition and the type of salts. The oregano essential oil nanoemulsions remained stable for several weeks in the absence of salts. Moreover, they were insensitive to tetrabutylammonium bromide, whereas similar to carvacrol emulsions, they exhibited a rapid phase separation and oiling-off in the presence of sodium chloride. On the other hand, high oleic sunflower oil (HOSO) and *p*-cymene emulsions remained stable in the presence of NaCl. Addition of 70 and 80% HOSO to the lipid phase of oregano EO and carvacrol, respectively, was found to be sufficient for the formation of emulsions with a high stability to 1.7 M NaCl. Hereby, the morphology of the oregano EO emulsions after 30 days of storage in the presence of NaCl was visualized using a transmission electron microscope. The determination of the surface load and area per surfactant molecule by interfacial tension (IFT) measurements and quartz crystal microbalance with dissipation revealed the dehydration of the polyoxyethylene groups of T80 in the presence of salt. The thickness of the T80 adsorbed layer onto solid hydrophobic and hydrophilic surfaces was significantly lower ($p < 0.05$) in the presence of sodium chloride. It is hypothesized that a combination of Ostwald ripening and coalescence due to an IFT increase and dehydration was responsible for the instability of the emulsions containing the more polar oregano EO and carvacrol in the presence of salt. The results obtained in this study could be useful for the formulation of essential oil nanoemulsions in the presence of salts applicable in food, pharmaceutical, and personal care products.



INTRODUCTION

Essential oils (EOs) are complex mixtures of organic compounds including volatile and nonvolatile compounds isolated from different parts of aromatic plants.¹ Recently, these components have attracted considerable interest due to distinct features such as their antioxidant, antimicrobial, anticancer, and antiradical activities^{2–6} in a broad category of products (e.g., food, medicinal, health care, and beauty products).⁷ Typically, the hydrophobic compounds need to be encapsulated within a colloidal system to protect them from degradation, to increase their bioavailability, to control their release, and to inhibit the possible impact on consumer perception.⁸ A vast variety of encapsulation systems have been introduced including conventional emulsions, microemulsions, nanoemulsions, solid particles, and liposomes.⁹ Oil-in-water nanoemulsions are finding an increasing interest as a delivery system over conventional emulsions because of their remarkable characteristics such as higher physicochemical stability, transparency, and higher bioavailability.^{10–12} Generally, different parameters may affect the final stability of the emulsions. For instance, Ostwald ripening (OR), the mass

transfer of oil molecules from smaller to larger droplets due to the difference in chemical potential within the droplets, is widely reported as an instability phenomenon in nanoemulsions.^{13,14}

Sodium chloride salt as a common functional ingredient is added to a lot of food products to improve their organoleptic and preservation characteristics. On the other hand, salt can induce detrimental impacts on the stability of emulsions. For instance, we surprisingly found in our previous study that rapid phase separation and oiling-off were observed when oregano essential O/W nanoemulsions were exposed to NaCl.¹⁴ In that study, polysorbate 80, a nonionic surfactant commercially available as Tween 80 (T80), was used to emulsify oregano essential oil droplets. Due to the absence of functional charged groups, T80 is mostly believed to be resistant to ionic strength variations. It has indeed been shown in several studies that the NaCl concentration did not have a considerable effect on the

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physicochemical stability of emulsions with no appreciable size variation when T80 was used as a surfactant.^{12,15} We also showed that nanoemulsions containing sunflower oil in the lipid phase remained stable with no size variation in the presence of up to 2 M NaCl.¹⁴ To the best of our knowledge, much less is known about the mechanism behind the influence of salt on the stability of essential oil nanoemulsions when T80 is utilized. For instance, Tadros¹⁶ reported the instability of nanoemulsions stabilized by polyethylene oxide-based surfactants in the presence of high electrolyte concentrations with no clear explanation. Thus, in this work, an attempt was made to figure out the reason for the instability of essential oil nanoemulsions in the presence of NaCl. Hereby, oregano essential oil, as well as some of its main components (i.e., carvacrol, and *p*-cymene), was considered. The outcomes of this study are of particular interest for the formulation of essential oil nanoemulsions using T80 in the presence of salt applicable in food, beauty, and agrochemical products.

■ EXPERIMENTAL SECTION

Materials. Oregano essential oil extracted from top flowers of *Origanum compactum* was provided by Pranarôm (Belgium). The oil was utilized without further purification. Para-cymene (*p*-cymene), carvacrol, thymol, T80, *n*-hexane, acetonitrile, and sodium azide ($\geq 99.5\%$) were purchased from Sigma-Aldrich Co. (St. Louis, MO). High oleic sunflower oil (HOSO) containing 83% C18:1 was provided by Contined B.V. (Bennekom, The Netherlands). Sodium chloride was obtained from VWR PROLABO Chemicals (Belgium). Ultrapure water purified by a Milli-Q filtration system (0.22 μm) (Millipore Corp., Bedford, MA) was used for the analyses and preparation of all aqueous solutions.

Gas Chromatography–Mass Spectrometry (GC–MS). Gas chromatography–mass spectrometry (GC–MS) analysis of the oils was performed using an Agilent GC–MS (Agilent, Belgium) equipped with a DB-5 ms (0.25 mm \times 30 m \times 0.25 μm) column and a quadrupole mass analyzer (Hewlett Packard GC 6890, MSD 5793). The temperature profile was set after several trials to 60–64 °C at 1 °C/min, 64–107 °C at 2.5 °C/min, 107–117 °C at 1 °C/min, and 117–250 °C at 7 °C/min. Electron ionization detection with an energy of 70 eV was utilized for MS detection in a mass range of 50–5500 Da. Oregano essential oil, carvacrol, thymol, and *p*-cymene were dissolved in *n*-hexane (high-performance liquid chromatography (HPLC) grade) as a solvent in a ratio of 1:80 w/v. The injection volume was set to 1 μL and speed of 50 $\mu\text{L/s}$ with a split ratio of 10:1. The recorded mass spectra were analyzed using Chemstation (Agilent) software and Wiley-National Institute of Standards and Technology data library. The relative percentage contribution of individual components was calculated based on the obtained total signal area.

Stock Emulsion Preparation. A stock oil-in-water nanoemulsion was prepared as described by Sedaghat Doost et al.¹⁷ Briefly, a mixture of the oil phase (5% w/w) consisting either of oregano essential oil, carvacrol, *p*-cymene, hexane, or HOSO and T80 aqueous solution (0.5% w/v) containing 0.02% w/v sodium azide (as an antimicrobial agent) was pre-emulsified using a high shear blender (Ultra-Turrax, type S 50N, IKA-Werke, Germany). Then, the emulsion was passed 10 times through a Microfluidizer M-110S at 112 MPa at 25 °C cooled down by placing the heat exchanger coil into an ice water bath. The samples were stored at 4 °C for further experiments.

The influence of HOSO concentration was examined by homogenizing 5 g of the lipid phase, which consisted of variable ratios of either oregano essential oil, carvacrol, or *p*-cymene and HOSO, with 95 g of the aqueous phase.

The prepared stock emulsions (5 wt %) were finally diluted 1:1 (v/v) with either Milli-Q water or 3.4 M salt solution, containing either $\text{N}(\text{CH}_3)_4\text{Cl}$, NH_4Cl , CsCl , KCl , NaCl , CaCl_2 , KSCN , tetramethy-

lammonium bromide (TMAB), tetraethylammonium bromide (TEAB), or tetrapropylammonium bromide (TPAB). This means that the concentration of the oil phase, T80, and salt in the final sample became half of the original sample. The salt was added afterward to ensure that it did not influence the emulsification step. Due to the limited solubility of tetrabutylammonium bromide (TBAB) in water i.e., 600 mg/mL, this salt was directly added to the emulsion (in dry salt form, 548 mg/mL). Hence, all of the studied salts were present at the same molar concentration (1.7 M).

Emulsion Characterization. Droplet Size Determination. Dynamic light scattering was employed to measure the *z*-average mean droplet diameter of the emulsions using a photon correlation spectrometer (model 4700, Malvern Instruments, U.K.) at a scattering angle of 150° at 25 °C. The emulsions were diluted prior to analysis with an appropriate salt solution or water to avoid multiple scattering, and the proper refractive indices and viscosity values^{18,19} of the diluting solutions were applied. The light intensity correlation function was analyzed based on the multimodal method, whereas the *z*-average diameter was obtained by cumulant analysis. The reported droplet size is the mean of at least two independent measurements.

Microstructure Characterization. Oregano EO emulsion (containing 70% HOSO in the lipid phase) diluted in 1.7 M NaCl, which was stored for 30 days, was further diluted (500 times in NaCl solution) prior to visualization. Negative staining was done as follows: 2 μL of the sample was applied onto a formvar-coated copper single slot grid (Agar Scientific, Standard, U.K.) and air-dried under a fume hood at ambient temperature covered by a glass dish. The grids were negative-stained by placing them on top of a drop of 1% aqueous uranyl acetate for 1 min after which the surplus was removed by holding a filter paper against the edge of the grid. The grid was ready to be studied with a transmission electron microscope (TEM). Photographs were recorded using a JEOL JEM 1010 (Jeol, Ltd, Tokyo, Japan) transmission electron microscope equipped with a Veleta side-mounted CCD camera (EMSYS GmbH, Muenster, Germany).

Total Organic Carbon (TOC) Measurement. The overall aqueous solubility of the studied oils was determined using a total organic carbon (TOC) analyzer (Shimadzu 5000, Shimadzu Scientific Instruments Inc., Japan). Samples with variable oil-to-water ratios (1.25–10% w/v) were prepared in the absence and presence of 1.7 M sodium chloride. A range of oil-to-aqueous-phase ratios was selected to ensure that minor compounds of oregano essential oil were also dissolved. Several incubation times were tested (1–24 h); there was no considerable variation in the solubility after 5 h, indicating that the oil/aqueous phase equilibrium was reached. The mixtures were left to be stirred for 5 h at 20 rpm using a rotational stirrer (SB3, Stuart, U.K.) at 20 °C. To separate the oil phase from the aqueous phase, the samples were centrifuged for 20 min at 3076g at 20 °C. An amount of the transparent aqueous phase was gently sucked out from the bottom of the tubes and diluted with an appropriate solvent. The concentration of carbon was determined by comparison with a calibration curve (10–100 ppm). The reported TOC concentration was a mean of three injections, whereas washing with a suitable solvent was performed four times prior to analyses. The obtained data were fitted to the Setschenow equation²⁰

$$\log(S_0/S) = KC \quad (1)$$

where *S* and *S*₀ are the solubilities of the compounds in aqueous sodium chloride solution and pure water, respectively; *K* represents the Setschenow constant; and *C* represents the salt concentration.

The aqueous solubilities of pure carvacrol ($\text{C}_{10}\text{H}_{14}\text{O}$), thymol ($\text{C}_{10}\text{H}_{14}\text{O}$), and *p*-cymene ($\text{C}_{10}\text{H}_{14}$) were calculated from the total organic carbon content (ppm) based on the molecular formula of individual compounds and the number of their carbon atoms.

High-Performance Liquid Chromatography Analysis. The concentrations of thymol and carvacrol, as two major components of oregano essential oil, were determined by high-performance liquid chromatography (HPLC, Agilent, Belgium) with a C18 kinetex (Phenomenex) column (150 mm \times 4.60 mm, particle size = 2.6 μm ,

and pore size = 100 Å) using a UV–vis detector (Agilent G1315B Diode Array) at 280 nm. A total of 10 μL of the aqueous phase, collected from Oregano essential oil (0.625 and 10% w/v), and an aqueous phase mixture prepared in a manner similar to that in the previous section, were injected with 0.5 mL/min flow rate. The following acetonitrile/water gradients were used: 0–15 min 50:50, 15–15:10 min 90:10, 15–18:10 min 90:10, and 18–24 min 50:50. The HPLC spectrum was analyzed using Chemstation (Agilent) software.

Quartz Crystal Microbalance with Dissipation (QCM-D). The influence of NaCl and T80 concentration on the adsorption behavior of T80 on two mimicking surfaces was studied using the quartz crystal microbalance with dissipation (QCM-D) technique. Bare silica sensors were used as model hydrophilic surfaces, whereas gold sensors coated by an undecanethiol monolayer are hydrophobic.

The thickness of the T80 adsorbed layer was obtained using the Q-Sense Dfind software (1.2.1., 2019) based on the Sauerbrey equation

$$\Delta m_f = -C\Delta f \quad (2)$$

where Δf is related to the mass changes (Δm_f) and C is expressed as a proportionality constant ($17.7 \text{ ng cm}^{-2} \text{ Hz}^{-1}$) that depends on the properties of the quartz and explains the sensitivity of the instrument to the mass changes. The specific details about the coating of gold sensors and calculations can be found in Sedaghat Doost et al.¹⁴

Interfacial Tension Determination. The influence of sodium chloride on the interfacial tension (IFT) between HOSO and T80 solution interfaces was studied using an automated drop shape tensiometer (TrackerTM, Teclis, I.T.C., France). The calibration of the tensiometer was performed as described by the manufacturer prior to each series of measurements. To be sure about the performance and cleaning procedure of the equipment, the surface tension (ST) of Milli-Q water–air was monitored ($71.5 \pm 1.0 \text{ mN/m}$ at 25°C). As density values have a major impact on these measurements, a water bath was connected to the cuvette holder to keep the temperature constant at 20°C . The measurements were conducted in duplicate, and the data were expressed as mean \pm standard deviation. The density of the oils as well as of the T80 aqueous solutions in the range of $10\text{--}45^\circ\text{C}$ was measured using an Anton-Paar density meter (DMA 5000). The equipment was calibrated using Milli-Q water.

The surface load (Γ), i.e., the amount of the surfactant per unit area, was calculated from the interfacial tension (γ) profile using the Gibbs equation

$$\Gamma = -\frac{1}{RT} \frac{d\gamma}{d \ln C} \quad (3)$$

where R is the universal gas constant, T is the absolute temperature, and C is the concentration of surfactant in moles per liter. The area (A) that each surfactant molecule occupies was also calculated as follows

$$A = \frac{1}{\Gamma N_A} \quad (4)$$

where N_A is expressed as Avogadro's number. The critical micelle concentration (CMC) was calculated from the intersection point of the linear regression based on the points with the highest slope and on the plateau region of the IFT profile, indicated in red and blue (Figure 8), respectively.

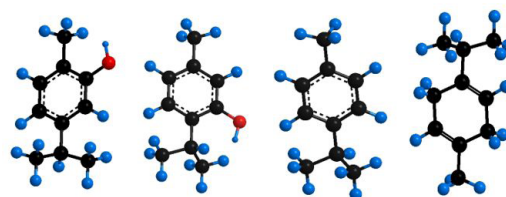
Statistical Analysis. The results are reported as mean \pm standard deviation. The statistical analyses were performed using SPSS software. ANOVA, one-way analyses were done to compare the significant differences between the means.

RESULTS AND DISCUSSION

The chemical constituents of the oregano essential oil (*O. compactum*) used in this study were differentiated using GC–MS analysis, and the most abundant compounds along with some three-dimensional (3D) chemical structures (drawn by ChemDraw software) are given in Table 1. Different

Table 1. Major Chemical Compounds (Representing at Least 1%) of Oregano Essential Oil Extracted from the Top Flowers of *Origanum compactum*^a

Compound	Relative amount (%)
carvacrol	51.67
thymol	16.50
p-cymene	13.48
γ -terpinene	9.48
α -terpinene	1.50
β -caryophyllene	1.30
delta-3-carene	1.09
Sum	95.02



Carvacrol Thymol p-cymene γ -terpinene

^aIn the 3D sketches, carbon, hydrogen, and oxygen are represented by black, blue, and red, respectively.

monoterpene and sesquiterpene hydrocarbons were identified. Among these, carvacrol (monoterpenoid, 52%), thymol (monoterpenoid, 17%), *p*-cymene (terpene, 13%), and γ -terpinene (monoterpene, 9%) represented larger peak areas compared to the peaks of other constituents, which is in line with previous studies.^{21,22} This indicates that oregano plant oil is a rich source of functional compounds, including carvacrol and thymol exerting antimicrobial and antioxidant properties.

Impact of Salt and Oil Type on the Stability of Nanoemulsions. Screening trials revealed that nanoemulsions carrying oregano essential oil diluted in Milli-Q water remained stable with a homogenous and opaque appearance, whereas these nanoemulsions were sensitive to the presence of sodium chloride. In Figure 1, it is evident that the emulsions were highly susceptible to phase separation and finally oiling-off in the presence of salt. Dilution of the emulsions obviously results in a decrease in the surfactant concentration and hence



Figure 1. Influence of different diluted solutions on the stability of 2.5% oregano essential oil nanoemulsions: 1:1 (v/v) dilution in distilled water (blank) was compared to 3.4 M NaCl and 0.5% T80 and 3.4 M NaCl (pictures after 1 h at 20°C).

an increase in interfacial tension.²³ To examine whether changes in the surfactant concentration upon dilution were responsible for the observed instability, the nanoemulsions were also diluted with a solution containing 0.5% T80 and 3.4 M NaCl (to keep the final surfactant concentration constant). However, the emulsions broke down even when the diluting solution contained the same surfactant concentration as the stock emulsion.

We hypothesized that the instability of the oregano EO emulsions might be explained from the behavior of its major components. Thus, pure carvacrol and *p*-cymene nanoemulsions were prepared. It is noteworthy to mention that despite the fact that oregano comprised a considerable thymol content, the emulsification of this compound was impossible due to its crystalline nature. Similar to oregano EO emulsions, the nanoemulsions containing carvacrol presented a severe instability when exposed to sodium chloride, whereas dilution into distilled water did not break down the colloidal dispersion. When the lipid phase of the nanoemulsions contained 100% *p*-cymene, no visible oiling-off took place within a few hours of storage, but creaming was observed, followed by the presence of oil droplets at the surface of the samples. Hence, the instability kinetics was largely slowed down. In a next series of experiments, HOSO was added to the lipid phase at different concentration levels to establish to what extent the oiling-off in the presence of salt could be inhibited. Size distribution analyses and visual appearance assessments displayed that replacing oregano EO and carvacrol to a certain amount by HOSO could suppress the extreme instability (Figures 2 and 3a). While the addition of 60% HOSO to oregano EO emulsions did not inhibit droplet size growth after NaCl addition, a 70% HOSO content in the lipid phase was capable to retard appreciably ($p < 0.05$) the size increase of these NaCl-containing emulsions under storage at 20 °C for 30 days (Figure 2a). The morphology and presence of the oregano EO nanoemulsions containing 70% HOSO in the lipid phase in the presence of 1.7 M NaCl after 30 days kept at 20 °C was studied using TEM (Figure 2b). It was observed that the oil droplets retained their spherical shape. This means that 70% HOSO was enough to prevent the destabilization of oregano EO emulsions in salt solution.

Carvacrol emulsions carrying 80 wt % HOSO in the oil phase had initially a droplet size growth during the first 5 days of storage, which was further leveled-off to day 30 (205 nm) with no sign of instability. Conversely, similar nanoemulsions with no added NaCl exhibited no noticeable ($p > 0.05$) mean size variation over incubation (Figure 4a). The initial increase was most probably due to Ostwald ripening (OR), which was suppressed due to the reduced transfer of carvacrol molecules in the presence of HOSO. *p*-cymene, on the other hand, needed only 10% HOSO to retain its long-term stability with no considerable mean droplet size changes (Figures 3 and 4b).

In a study performed by Chanamai et al.,²⁴ the effect of the oil polarity and surfactant type on the droplet growth in emulsions was examined. They found that emulsions containing a lipid phase with low polarity and water solubility, in that case, hexadecane, were stable. By contrast, if the oil phase has a low polarity but a higher water solubility (such as decane), the emulsions were susceptible to OR but resistant to coalescence. An emulsion prepared by an oil, which has a high polarity and water solubility (decanol), exhibited droplet growth depending on the surfactant type. They attributed this droplet size increase to the low interfacial tension between the

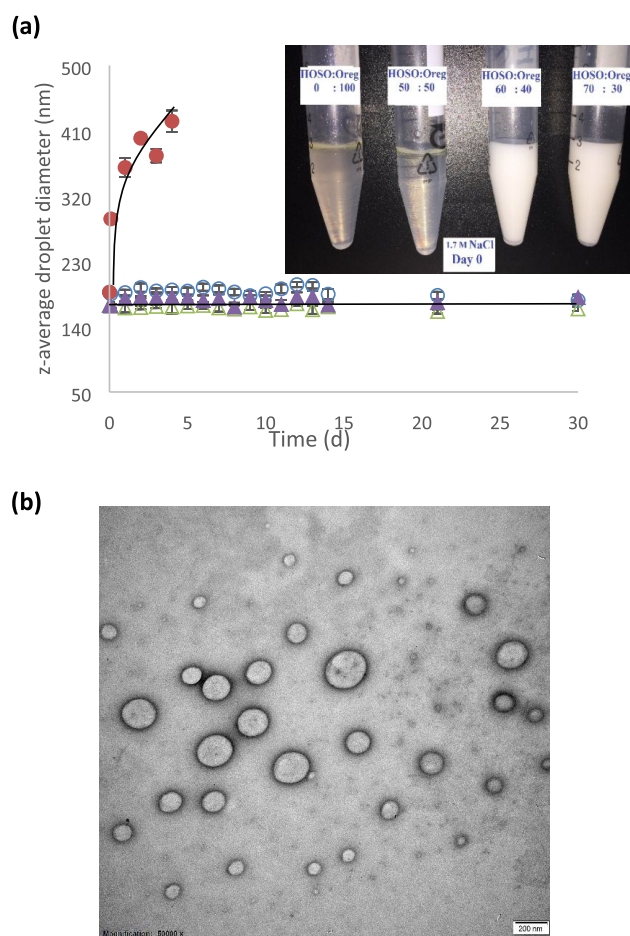


Figure 2. (a) Z-average mean droplet size variation of 2.5% oregano essential oil-in-water nanoemulsions containing 60% (circles) and 70% (triangles) high oleic sunflower oil (HOSO) in the absence (empty symbols) and presence (filled symbols) of 10% (1.7 M) sodium chloride. (b) TEM photographs of oregano O/W containing 70% HOSO in the presence of 1.7 M NaCl after 30 days of storage at 20 °C.

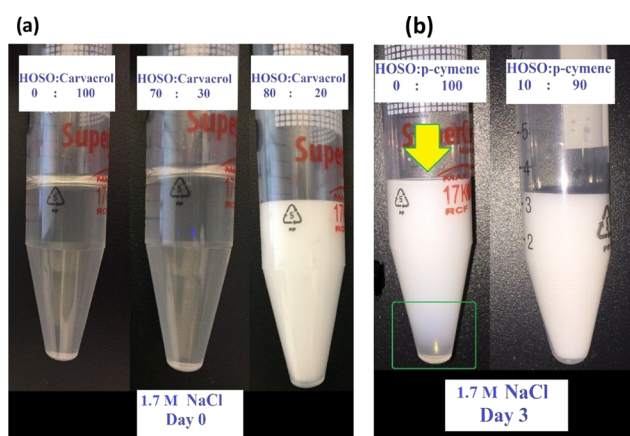


Figure 3. Visual appearance of (a) carvacrol and (b) *p*-cymene oil-in-water nanoemulsions containing 0, 70, and 80 and 0 and 10 wt % high oleic sunflower oil (HOSO), respectively, in the presence of 1.7 M NaCl.

two solvents with some degree of mutual solubility (decanol and the aqueous phases), resulting in a combination of coalescence and OR.

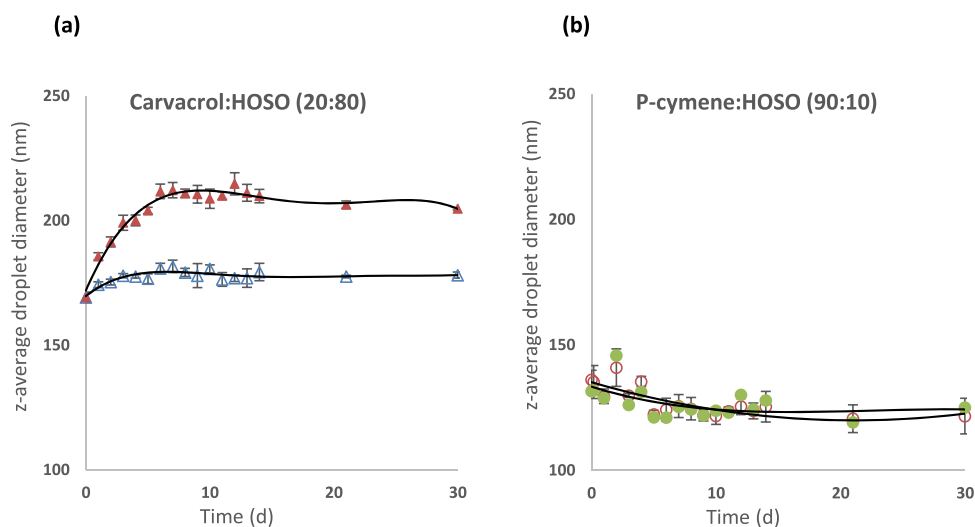


Figure 4. Mean droplet size variation of (a) carvacrol nanoemulsions containing 80 wt % high oleic sunflower oil (HOSO) and (b) *p*-cymene containing 10 wt % HOSO in the absence (empty symbols) and presence (filled symbols) of 1.7 M sodium chloride.

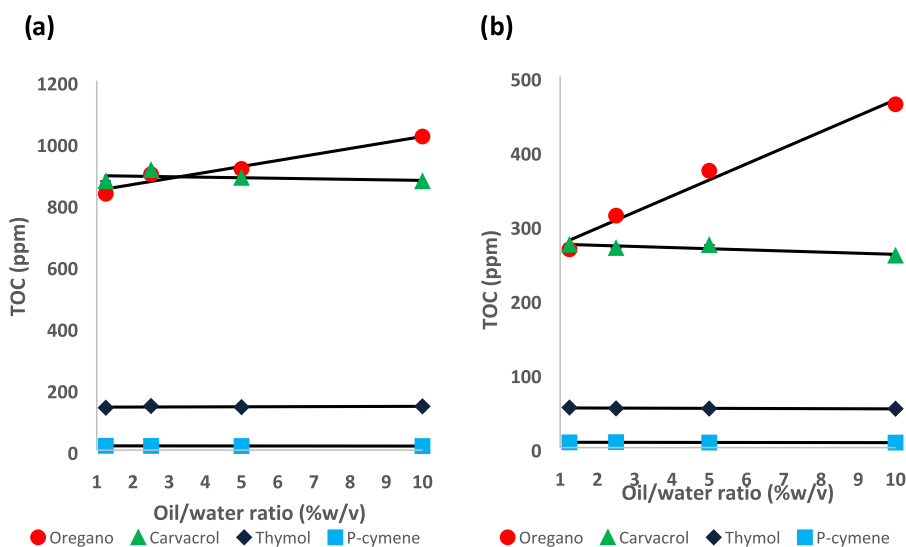


Figure 5. Comparison of the overall solubilities of oregano essential oil (red circle solid) and its major constituents [carvacrol (green triangle-up solid), thymol (navy-blue tilted square solid), and *p*-cymene (blue box solid)] in an aqueous solution containing (a) 0 and (b) 1.7 M NaCl at 20 °C.

Thus, it is thought that even though T80 is expected to be resistant to salt-induced aggregation at a high salt content, other instability mechanisms may play a role, which will be affected by the oil type. It was hypothesized that the initial fast OR due to the significant aqueous solubility of oregano EO or carvacrol may promote creaming and thereby promote coalescence leading to the oiling-off. OR occurs because the Laplace pressure results in a higher chemical potential of the smaller droplets of the dispersed phase than of the larger ones. This is the driving force for the movement of the dispersed phase molecules from smaller droplets to larger droplets.¹³ According to the Lifshitz–Slyozov–Wagner (LSW) theory, the rate of OR follows from eq 5

$$r^3 - r_0^3 = \omega t = \frac{4}{9} \alpha S(\infty) D t \quad (5)$$

where the change of the droplet radius r as a function of time (t) is related to the solubility of the solute in the bulk phase (S), the translational diffusion coefficient (D), and α , which is

$$\alpha = 2\gamma V_m / RT \quad (6)$$

where γ is the interfacial tension and V_m is the molar volume of the solute.

Our results suggested that emulsions of oils with a higher polarity and hence more tendency to dissolve in the aqueous phase were less resistant to the presence of NaCl. This was surprising because, from a physical chemistry point of view, salt is expected to decrease the aqueous solubility of a compound through the well-known salting-out phenomenon. The overall solubilities of oregano essential oil and its major components at different oil-to-aqueous-phase volumetric ratios both in the presence and absence of 1.7 M sodium chloride were determined based on the amount of total organic carbon (TOC) in the aqueous phase (Figure 5). The solubility was not measured in the nanoemulsions because the droplet size plays a role in the solubility of the oil within the continuous phase. The results indeed presented that the solubility of oregano essential oil compounds generally decreased in the

presence of NaCl, which is in line with the salting-out effect. In the presence of Na^+ and Cl^- ions, the water molecules make a hydration shell around these ions. Hence, fewer water molecules are available to interact with the oil molecules, thereby decreasing the aqueous solubility. This effect was confirmed by fitting the obtained data to the Setschenow equation.²⁰ For instance, the Setschenow constant at an oregano-oil-to-water ratio of 1.25% was $0.051 (\% \text{ NaCl})^{-1}$, indicating that at a higher NaCl concentration, less oregano essential oil was soluble within the aqueous phase.

The analysis of the slope obtained from the fitted linear regression revealed that the oregano-oil-to-water ratio had a positive effect on the water solubility of oregano essential oil in the absence of salt: the slope was $19.5 \pm 13 \text{ ppm}/\%$ oregano essential oil. This is due to the increased dissolved amount of some minor compounds that do not reach their equilibrium concentration within the concentration range studied. This effect was more pronounced upon increasing the oregano/water ratio in the presence of 1.7 M salt ($21.6 \pm 9 \text{ ppm}/\%$ oregano essential oil). On the other hand, by increasing the amount of pure compounds, i.e., carvacrol, thymol, and *p*-cymene, in water (both in the absence and presence of NaCl), a constant water solubility was observed, indicating that the threshold water solubility of these compounds was already reached at the lowest studied oil/water ratio, and hence the organic molecules were in equilibrium with the aqueous phase.

In Figure 5, it seems that carvacrol determines the overall water solubility of oregano essential oil in the presence of a large excess of water. For instance, the aqueous solubility of both oregano EO and carvacrol at a ratio of 1.25% in the presence of salt was 270 ppm, whereas this value became 460 and 260 ppm, respectively, when the ratio of the organic phase to the NaCl aqueous phase was 10%. Therefore, it was concluded that at the lowest studied oil/water ratio, the solubility of oregano EO is to a large extent dominated by carvacrol as its major constituent, whereas at higher ratios, the dissolution of minor compounds will play a major role. To examine this, HPLC was employed to compare the organic composition of the aqueous phase at low (0.625% w/v) and high (10% w/v) oil-to-water ratios. The results suggested that the dissolved amount of carvacrol and thymol did not grow by increasing the ratio of oregano essential oil. On the other hand, some peaks related to minor components either increased or appeared at 10% oil, indicating more dissolution of these compounds in the aqueous phase (Figure 6).

Based on Figure 5, and considering the carbon content of the different compounds, the aqueous solubilities of carvacrol, thymol, and *p*-cymene in water were estimated to be 1.09, 0.17, and 0.02 g/L, respectively, which decreased considerably to 0.34, 0.07, and 0.01 g/L in the 1.7 M NaCl aqueous phase. These results of the solubility in the aqueous phase were in a reasonable agreement with previously reported outcomes.^{25–27}

According to the LSW theory for Ostwald ripening, the higher the water solubility of the dispersed phase, the higher the rate of OR would be. Thus, it seems that the reduction of the aqueous phase solubility of oregano essential oil by the addition of sodium chloride is less effective to reduce the OR rate as compared to possible changes in other parameters, such as the interfacial tension.

Adsorption Characteristic at Liquid–Solid Surfaces.

The adsorption at a liquid–solid interface of T80 molecules dissolved in an aqueous phase containing 0 or 1.7 M sodium chloride on undecanethiol-coated gold sensors and bare silica

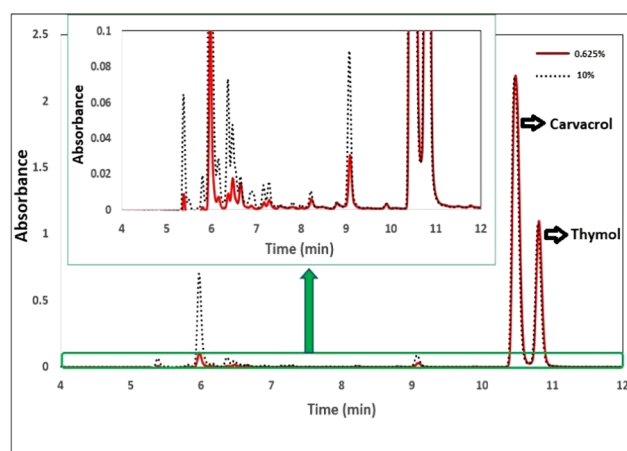


Figure 6. HPLC spectra of organic compounds present in the aqueous phases of two oil–water mixtures (ratio = 0.625 and 10%) in the absence of NaCl.

sensors was studied using QCM-D. Two different sensor surfaces were utilized to evaluate the possible effect of salt addition on the adsorption of T80 molecules on hydrophilic and hydrophobic surfaces. The studied concentrations ranged from below the T80 CMC (i.e., 0.01 mM) to higher values. While a thicker adsorbed layer might be expected at higher T80 concentrations due to the adsorption of more molecules, Figure 7 indicated that the thickness of the adsorbed layer did not significantly increase at higher concentrations, showing that the adsorption continued only until the surface of the sensor was fully covered, which was already the case at the lowest concentrations considered. Interestingly, the presence of salt had a significant effect on the thickness of the adsorbed layer: a thinner layer of T80 deposited on the hydrophobic surface of the coated gold sensors, when it was in contact with a concentrated salt solution. For instance, for 2.5% T80 solution, the thickness decreased from 3.2 ± 0.2 to 2.6 ± 0.1 nm in the presence of 1.7 M NaCl. The T80 concentration variation did not have a significant ($p < 0.05$) influence on the thickness of the deposited layer. Chemically, T80 is mainly composed of the monooleate ester of ethoxylated sorbitan moieties. It seems that sodium chloride removed water molecules and hence decreased the hydration of the hydrophilic head groups. This may partly explain the instability of the nanoemulsions stabilized by T80: the droplets may come closer to each other as they have a more compact head group upon addition of sodium chloride and hence the steric stabilization is less efficient. Schick²⁸ studied the effect of electrolyte concentration on the surface tension variation of polyoxyethylene nonylphenol nonionic surfactants. He ultimately postulated a mechanism for the salting-out effects of the head group of polyoxyethylene nonylphenols. His hypothesis was that when the concentration of electrolyte increases, removal of the hydrogen-bonded water molecules from the ether oxygens of polyoxyethylene groups would occur. This could be applicable to what is happening to the T80 molecules at the interface of oregano EO nanoemulsions in the presence of sodium chloride. However, this hypothesis can only partially explain the observed instability as by changing the oil phase to HOSO the emulsions retained their stability.

To evaluate the effect of sodium chloride on the hydrogen bonding ability of the T80 head groups, bare silica sensors were used to mimic a hydrophilic surface. A similar behavior

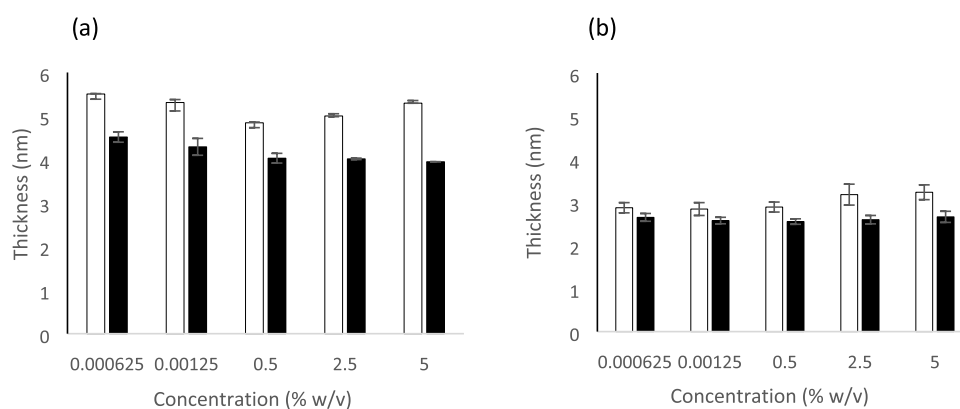


Figure 7. Influence of salt (NaCl) on the adsorbed thickness of Tween 80 at different concentrations (0.000625–5%) in water (empty) and 1.7 M salt solution (filled) on (a) silica- and (b) undecanethiol-coated gold sensors.

was observed in the case of silica sensors in which salt reduced again considerably ($p < 0.05$) the adsorption thickness of T80; e.g., for 2.5% T80, the thickness was reduced from 5.00 ± 0.05 to 4.02 ± 0.02 nm. Surprisingly, the thickness of the deposited layer on top of the silica sensors was almost double irrespective of the aqueous phase condition. This could be attributed to the way that T80 molecules adsorb in which the first layer of T80 adsorbs to the hydrophilic surface via their head groups, whereas the tails are oriented toward the bulk phase. A second layer then assembles on top of this hydrophobized surface with the tails coating the alkyl chain network of the already adsorbed layer.

Interfacial Tension Changes. The influence of the addition of sodium chloride on the interfacial tension (IFT) between the oil and the aqueous phase was evaluated. The drop shape analysis method is commonly used to determine the IFT at the interface of two immiscible liquids, in this case, the aqueous solution and the oil phase. It calculates the IFT based on the Laplace pressure changes by analyzing the drop shape upon formation of one phase into another phase considering that there is sufficient density difference between the two phases. If an oil droplet is formed into an aqueous phase containing surfactant molecules, the molecules start to move to the surface of the oil droplet and the IFT starts decreasing until the surface becomes saturated. This process may take a few minutes depending on the nature and chemical composition of the oil and emulsifier and the viscosity of the bulk phase. In this study, several attempts were made to determine the IFT at the interface of oregano essential oil and surfactant solution. However, the formation of Laplacian droplets after injection of oil into the aqueous phase seemed impossible and a track of dissolving oil droplets within the surfactant solution was observed. This could be explained from the relatively high solubility of the oil in the water, which is further increased in the presence of T80 micelles through micellar solubilization, and from the resulting ultralow interfacial tension, which has been typically reported for essential oils.²⁹ Surprisingly, IFT determination using the spinning drop technique, which can measure ultralow IFT values, was also not successful, indicating that dissolution of the oil molecules within the aqueous phase made the measurements critical.

On the other hand, IFT measurements using HOSO as a triglyceride with very low water solubility were feasible. The results are shown in Figure 8. T80 in both aqueous phases presented somewhat similar trends. Nevertheless, the IFT

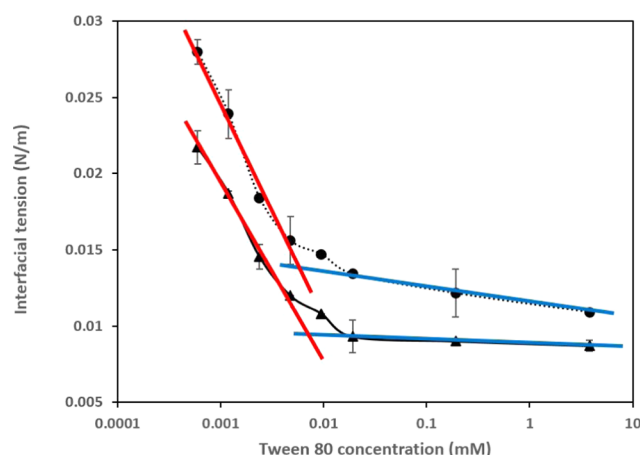


Figure 8. Interfacial tension evolution at the interface of high oleic sunflower oil (HOSO) and aqueous solutions containing variable concentrations of Tween 80 in the absence \blacktriangle and presence \bullet of 1.7 M sodium chloride at 25 °C.

profile of the T80 solution containing sodium chloride showed higher values than the IFT profile of T80 in Milli-Q water. This may explain the results of droplet size evaluation where the emulsions containing HOSO and *p*-cymene with low water solubility remained stable. Conversely, carvacrol and oregano EO emulsions with the highest solubility in water showed a severe oiling-off upon NaCl dilution (not in distilled water).

Typically, by addition of more emulsifier, the IFT drops considerably up to a concentration, known as the CMC, above which the IFT hardly changes, showing that the surface of the oil droplets has become saturated. The CMC of T80 in the aqueous phase with or without NaCl was identical, i.e., 0.01 ± 0.00 mM. This outcome was in good agreement with work performed by Qazi et al.,³⁰ who reported no changes in the CMC of T80 in the presence of NaCl.

Linear regression on the results obtained for the lowest four concentrations showed that the surface load (Γ) was 2.8 ± 0.1 and 3.3 ± 0.4 mg/m² for T80 in the absence and presence of 1.7 M NaCl, respectively. This indicated that more surfactant molecules were present at the oil–water interface when sodium chloride is present in the aqueous phase. The results are in agreement with the outcomes previously reported by Yang et al.³¹ The higher surface load in the presence of NaCl can be attributed to the fact that dissociation of NaCl molecules within water breaks the interaction of water

molecules with the POE head groups of T80 and therefore dehydration of these groups occurs. This favors the accumulation of the surfactant molecules from the aqueous phase toward a more hydrophobic environment. The area (A) that each surfactant molecule occupies was also determined and was 79.0 ± 3.0 and $66.4 \pm 8.8 \text{ \AA}^2$ for T80 in the absence and presence of 1.7 M NaCl, respectively. The former result was consistent with the outcome of previous work by Yang et al.³¹ This means that a T80 molecule dissolved in salt solution requires less space to become adsorbed at an interface than when there is no salt present.

However, the instability of the oregano nanoemulsions is not solely interpretable by dehydration of the T80 molecules. As the HOSO emulsions stabilized by T80 retained their stability with no droplet size variation by addition of 1.7 M NaCl, this is an indication that this instability is unique for the studied oils. This shows that the instability was most probably started by OR leading to the growth of the droplet size followed by creaming, which in its turn promoted coalescence. In fact, the presence of salt provides conditions that may favor coalescence, i.e., the dehydration of the T80 head groups resulting in a thinner surfactant protection layer on the surface of the oil droplets.

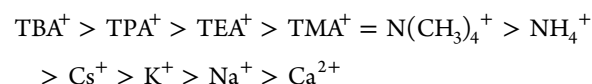
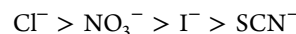
Binks et al.³² studied the effect of salt concentration on the creaming and coalescence stability of heptane oil-in-water emulsions stabilized by sodium bis(2-ethylhexylsulfosuccinate) (AOT). They concluded that in systems containing a high NaCl concentration, which have ultralow interfacial tension, coalescence is responsible for the instability of the emulsions due to the decreased repulsion by screening the AOT charges and easier deformation of the droplets when they come in close vicinity of each other. Despite the fact that AOT is an anionic surfactant and NaCl suppresses the electrostatic repulsion by interaction with the charged group ($-\text{SO}_3^- \text{Na}^+$) of this emulsifier and thereby induces flocculation followed by enhanced coalescence, this hypothesis might be generalized to oregano EO nanoemulsion instability.³³

The overall solubility results suggested that sodium chloride reduced the solubility of oregano essential oil and its main compounds in the aqueous phase. Based on these observations, eq 5 seems to indicate that the addition of salt reduces the OR rate. However, our results indicate the opposite. According to eq 6, the OR rate is also directly proportional to the interfacial tension at the liquid/liquid interface: increasing the IFT might promote a higher rate of droplet coarsening.

We also examined this hypothesis by diluting 5% hexane-in-water emulsions (stabilized using 0.5% T80) in water and salt solution separately. It was expected that hexane with a shorter hydrocarbon chain length is relatively more water soluble than hydrocarbons with a longer chain or triglycerides and is therefore more prone to OR. Blank emulsions in water were separated with a cream layer, which is due to the lower density of hexane (0.66 g/mL) than water. However, this layer did not show any sign of oiling-off. The emulsions had severe phase separation and oiling-off (after one day) in the presence of sodium chloride. This implies that NaCl largely accelerated the OR of the hexane emulsions.

As dehydration due to the competition for water between polyethylene oxide and dissolved salt was expected to play a role, a Hofmeister salt series was selected to evaluate the influence of the type of the salt on the visual appearance of the oregano O/W emulsions. The Hofmeister series considers two types of ions, which influence the solubility of solutes by

changes in the structure of water molecules contacting the solute: salting-in (hydration) and salting-out (dehydration) ions.³⁴ The order of the anions and cations in the Hofmeister series is as follows



In this series, hydration increases from left to right for anions and vice versa for cations. Interestingly, the emulsions broke down rapidly with a thick oil layer on top and an aqueous phase at the bottom in the presence of most studied salts (Figure 9). However, the speeds of the separation and oiling-off were different depending on the type of salt.

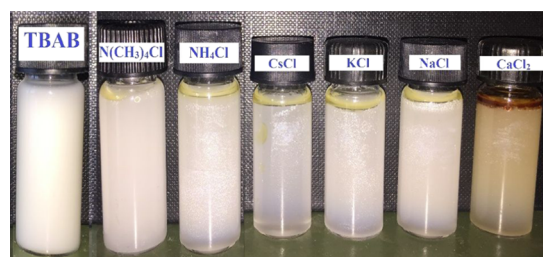


Figure 9. Influence of salt type on the stability of T80-stabilized oregano essential oil emulsions at 20 °C.

Previously, the influence of sodium chloride as a lyotropic salt and tetrabutylammonium bromide (TBAB), a hydrotropic salt, on the stability of silicone oil emulsified using nonionic surfactants was investigated by Binks and Dong.³⁵ They concluded that the addition of NaCl led to a reduction of the phase inversion temperature (PIT) of C_{12}E_5 , whereas TBAB increased the PIT. It was thought that the NaCl ions compete with the water hydrogen-bonded to ethyleneoxide moieties of the surfactant via ion-dipole forces and thereby reduce the PIT. Unlike the silicone emulsions diluted with 0.01 M NaCl that became very unstable above 45 °C, the addition of 0.1 to 1 M TBAB showed a PIT increase to > 70 °C, indicating the salting-in of the surfactant.³⁵ We also examined this hypothesis by adding 548 mg/mL TBAB into the oregano emulsions. Surprisingly, the emulsions did not exhibit any sign of instability after 5 days of storage at 20 °C with an opaque and homogenous appearance.

It has been shown that the surface tension is correlated with the solvation energy of ions in the aqueous phase leading to a Hofmeister series.³⁶ Among the salts examined in this work, only TBAB would decrease the surface tension (ST), whereas the addition of the other salts has been found to increase the aqueous surface tension at different levels.^{37,38} In fact, the surface tension is a manifestation of differences in the water binding: more strongly water-binding cations lead to stronger destruction of the water structure. Consequently, cations that more strongly bind water increase the IFT, whereas TBAB rather reduces the IFT as it is somewhat surface active. For instance, the ST of water increased by 1.73 mN/m by addition of 1 M NaCl in comparison to 1 M NaSCN, which caused only a 0.5 mN/m increase in ST. The lower surface or interfacial tension could explain the higher stability of the emulsions exposed to TBAB. It has been previously found by Michler et al.³⁹ that unlike NaCl as an inorganic salt, which increases the

IFT, organic tetraphenyl borate anions lower the IFT. It is also noteworthy to highlight that TBAB does not form micelles.⁴⁰

Quaternary salts with different alkyl chains (TMAB, TEAB, and TPAB) were also tested. They can either increase or decrease the ST of water, thereby influencing the stability of oregano emulsions. The surface activity of the quaternary ammonium compounds increased by increasing the length of their alkyl chains. It was observed that oregano emulsions diluted in TMAB had oiling-off after a few hours, but, interestingly, the emulsions remained stable considerably longer when TEAB (after 1 days) and TPAB (after 3 days) were present. This could be attributed to the change in the ST induced by these salts as has been shown by Patel et al.,⁴¹ where the ST was reduced by TEAB, TPAB, and TBAB, whereas TMAB addition led to an increase in the ST of the aqueous phase. Therefore, the instability of the nanoemulsions is synergistically increased by both the water solubility of the dispersed phase and the addition of salt that reduces the hydrophilicity of T80 molecules.

SUMMARY AND CONCLUSIONS

The influence of salt on the physicochemical stability of emulsions of oregano essential oil or some of its main components, stabilized using the nonionic surfactant Tween 80, was studied. In the absence of salts, these emulsions remained stable for several weeks. Upon salt dilution, we found that the oil type had a substantial influence on the stability of the emulsions. Oregano essential oil and carvacrol emulsions showed a rapid oiling-off after addition of NaCl, whereas *p*-cymene, as well as sunflower oil emulsions, remained stable. This instability was found to be due to OR leading to creaming due to the droplet size growth, which further promoted coalescence in the later stages. The results suggested that suppression of OR by addition of a poorly water-soluble oil, such as HOSO, to the dispersed phase can inhibit this destabilization. Although surface load results obtained by interfacial tension measurements and adsorption characteristics of T80 on a solid surface showed the dehydration of the head groups upon NaCl dilution, the polarity of the oil and presumably small changes in the (very low) interfacial tension values between the polar oil and water contributed to this instability. Addition of TBAB to oregano EO emulsions indeed showed no noticeable changes, which was most probably related to the IFT reduction, delaying the OR. As there is a great demand for the utilization of essential oils in different products, this study provides theoretical and practical information for the design of an appropriate delivery system.

AUTHOR INFORMATION

Corresponding Author

*E-mail: Ali.sedaghatdoost@ugent.be. Phone: +32 (0)9 264 60 04.

ORCID

Ali Sedaghat Doost: [0000-0003-1090-8082](https://orcid.org/0000-0003-1090-8082)

Christian V. Stevens: [0000-0003-4393-5327](https://orcid.org/0000-0003-4393-5327)

Notes

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REFERENCES

- (1) Jobdeedamrong, A.; Jenjob, R.; Crespy, D. Encapsulation and Release of Essential Oils in Functional Silica Nanocontainers. *Langmuir* **2018**, *34*, 13235–13243.
- (2) Sedaghat Doost, A.; Devlieghere, F.; Dirckx, A.; Van der Meer, P. Fabrication of *Origanum compactum* essential oil nanoemulsions stabilized using Quillaja Saponin biosurfactant. *J. Food Process. Preserv.* **2018**, *42*, No. e13668.
- (3) Sedaghat Doost, A.; Van Camp, J.; Dewettinck, K.; Van der Meer, P. Production of thymol nanoemulsions stabilized using Quillaja Saponin as a biosurfactant: Antioxidant activity enhancement. *Food Chem.* **2019**, *293*, 134–143.
- (4) Terjung, N.; Löffler, M.; Gibis, M.; Hinrichs, J.; Weiss, J. Influence of droplet size on the efficacy of oil-in-water emulsions loaded with phenolic antimicrobials. *Food Funct* **2012**, *3*, 290–301.
- (5) Zamindar, N.; Bashash, M.; Khorshidi, F.; Serjouie, A.; Shirvani, M. A.; Abbasi, H.; Sedaghat Doost, A. Antioxidant efficacy of soybean cake extracts in soy oil protection. *J. Food Sci. Technol.* **2017**, *54*, 2077–2084.
- (6) Zdrorow, K. R.; Schiffman, J. D.; Elimelech, M. Biodegradable Polymer (PLGA) Coatings Featuring Cinnamaldehyde and Carvacrol Mitigate Biofilm Formation. *Langmuir* **2012**, *28*, 13993–13999.
- (7) Balzamo, G.; Willcock, H.; Ali, J.; Ratcliffe, E.; Mele, E. Bioinspired Poly(vinylidene fluoride) Membranes with Directional Release of Therapeutic Essential Oils. *Langmuir* **2018**, *34*, 8652–8660.
- (8) Sedaghat Doost, A.; Nikbakht Nasrabadi, M.; Kassozi, V.; Dewettinck, K.; Stevens, C. V.; Van der Meer, P. Pickering stabilization of thymol through green emulsification using soluble fraction of almond gum-Whey protein isolate nano-complexes. *Food Hydrocolloids* **2019**, *88*, 218–227.
- (9) McClements, D. J.; Jafari, S. M. General Aspects of Nanoemulsions and Their Formulation. In *Nanoemulsions*; Jafari, S. M., McClements, D. J., Eds.; Academic Press, 2018; Chapter 1, pp 3–20.
- (10) Jafari, S. M.; Assadpoor, E.; He, Y.; Bhandari, B. Re-coalescence of emulsion droplets during high-energy emulsification. *Food Hydrocolloids* **2008**, *22*, 1191–1202.
- (11) McClements, D. J.; Jafari, S. M. Improving emulsion formation, stability and performance using mixed emulsifiers: A review. *Adv. Colloid Interface Sci.* **2018**, *251*, 55–79.
- (12) Saberi, A. H.; Fang, Y.; McClements, D. J. Effect of Salts on Formation and Stability of Vitamin E-Enriched Mini-emulsions Produced by Spontaneous Emulsification. *J. Agric. Food Chem.* **2014**, *62*, 11246–11253.
- (13) Gupta, A.; Eral, H. B.; Hatton, T. A.; Doyle, P. S. Nanoemulsions: formation, properties and applications. *Soft Matter* **2016**, *12*, 2826–2841.
- (14) Doost, A. S.; Sinnaeve, D.; De Neve, L.; Van der Meer, P. Influence of non-ionic surfactant type on the salt sensitivity of oregano oil-in-water emulsions. *Colloids Surf., A* **2017**, *525*, 38–48.
- (15) Wu, M.-H.; Yan, H. H.; Chen, Z.-Q.; He, M. Effects of emulsifier type and environmental stress on the stability of curcumin emulsion. *J. Dispersion Sci. Technol.* **2017**, *38*, 1375–1380.
- (16) Tadros, T. Stabilisation of dispersions using a graft copolymer of hydrophobically modified polyfructose. *Colloids Surf., A* **2017**, *519*, 11–19.
- (17) Doost, A. S.; Dewettinck, K.; Devlieghere, F.; Van der Meer, P. Influence of non-ionic emulsifier type on the stability of cinnamaldehyde nanoemulsions: A comparison of polysorbate 80 and hydrophobically modified inulin. *Food Chem.* **2018**, *258*, 237–244.
- (18) Kestin, J.; Shankland, I. R. Viscosity of aqueous NaCl solutions in the temperature range 25–200 °C and in the pressure range 0.1–30 MPa. *Int. J. Thermophys.* **1984**, *5*, 241–263.
- (19) Tan, C.-Y.; Huang, Y.-X. Dependence of Refractive Index on Concentration and Temperature in Electrolyte Solution, Polar

Solution, Nonpolar Solution, and Protein Solution. *J. Chem. Eng. Data* **2015**, *60*, 2827–2833.

(20) Noubigh, A.; Abderrabba, M.; Provost, E. Temperature and salt addition effects on the solubility behaviour of some phenolic compounds in water. *J. Chem. Thermodyn.* **2007**, *39*, 297–303.

(21) Bouyahya, A.; Abrini, J.; Bakri, Y.; Dakka, N. Phytochemical screening and evaluation of antioxidant and antibacterial activities of *Origanum compactum* extracts. *Phytothérapie* **2017**, *15*, 379–383.

(22) Laghmouchi, Y.; Belmehdi, O.; Senhaji, N. S.; Abrini, J. Chemical composition and antibacterial activity of *Origanum compactum* Benth. essential oils from different areas at northern Morocco. *S. Afr. J. Bot.* **2018**, *115*, 120–125.

(23) Taylor, P.; Ottewill, R. H. The formation and ageing rates of oil-in-water miniemulsions. *Colloids Surf., A* **1994**, *88*, 303–316.

(24) Chanamai, R.; Horn, G.; McClements, D. J. Influence of Oil Polarity on Droplet Growth in Oil-in-Water Emulsions Stabilized by a Weakly Adsorbing Biopolymer or a Nonionic Surfactant. *J. Colloid Interface Sci.* **2002**, *247*, 167–176.

(25) Martins, M. A. R.; Silva, L. P.; Ferreira, O.; Schröder, B.; Coutinho, J. A. P.; Pinho, S. P. Terpenes solubility in water and their environmental distribution. *J. Mol. Liq.* **2017**, *241*, 996–1002.

(26) Steinbrecher, R.; Koppmann, R. Biosphäre und Atmosphäre: Bedeutung biogener Kohlenwasserstoffe. *Chem. Unserer Zeit* **2007**, *41*, 286–292.

(27) Svendby, T. M.; Lazaridis, M.; Tørseth, K. Temperature dependent secondary organic aerosol formation from terpenes and aromatics. *J. Atmos. Chem.* **2008**, *59*, 25–46.

(28) Schick, M. J. Surface films of nonionic detergents—I. Surface tension study. *J. Colloid Sci.* **1962**, *17*, 801–813.

(29) Arneodo, C.; Baszkin, A.; Benoit, J. P.; Fellous, R.; Thies, C. Interfacial studies of essential oil—water systems. *Colloids Surf.* **1988**, *34*, 159–169.

(30) Qazi, M. J.; Loefflerink, R. W.; Schlegel, S. J.; Backus, E. H. G.; Bonn, D.; Shahidzadeh, N. Influence of Surfactants on Sodium Chloride Crystallization in Confinement. *Langmuir* **2017**, *33*, 4260–4268.

(31) Yang, Y.; Leser, M. E.; Sher, A. A.; McClements, D. J. Formation and stability of emulsions using a natural small molecule surfactant: Quillaja saponin (Q-Naturale). *Food Hydrocolloids* **2013**, *30*, 589–596.

(32) Binks, B. P.; Cho, W. G.; Fletcher, P. D. I.; Petsev, D. N. Stability of Oil-in-Water Emulsions in a Low Interfacial Tension System. *Langmuir* **2000**, *16*, 1025–1034.

(33) Mitchell, D. J.; Ninham, B. W. Micelles, vesicles and microemulsions. *J. Chem. Soc., Faraday Trans. 2* **1981**, *77*, 601–629.

(34) Pleines, M.; Kunz, W.; Zemb, T.; Benczédi, D.; Fieber, W. Molecular factors governing the viscosity peak of giant micelles in the presence of salt and fragrances. *J. Colloid Interface Sci.* **2019**, *537*, 682–693.

(35) Binks, B. P.; Dong, J. Emulsions and equilibrium phase behaviour in silicone oil + water + nonionic surfactant mixtures. *Colloids Surf., A* **1998**, *132*, 289–301.

(36) Boström, M.; Kunz, W.; Ninham, B. W. Hofmeister Effects in Surface Tension of Aqueous Electrolyte Solution. *Langmuir* **2005**, *21*, 2619–2623.

(37) Mata, J.; Varade, D.; Ghosh, G.; Bahadur, P. Effect of tetrabutylammonium bromide on the micelles of sodium dodecyl sulfate. *Colloids Surf., A* **2004**, *245*, 69–73.

(38) Pegram, L. M.; Record, M. T. Hofmeister Salt Effects on Surface Tension Arise from Partitioning of Anions and Cations between Bulk Water and the Air–Water Interface. *J. Phys. Chem. B* **2007**, *111*, 5411–5417.

(39) Michler, D.; Shahidzadeh, N.; Westbroek, M.; van Roij, R.; Bonn, D. Are Antagonistic Salts Surfactants? *Langmuir* **2015**, *31*, 906–911.

(40) Tamaki, K. The Surface Activity of Tetrabutylammonium Halides in the Aqueous Solutions. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 38–41.

(41) Patel, J.; Varade, D.; Bahadur, P. Effect of tetraalkylammonium bromides on the micellar behaviour of ionic and non-ionic surfactants. *Indian J. Chem., Sect. A: Inorg., Phys., Theor. Anal. Chem.* **2004**, *43*, 715–721.