Characterization of novel nanoemulsions, with improved properties, based on rosemary essential oil and biopolymers.

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Abstract

BACKGROUND: Nowadays, it is of great interest to develop stable and sustainable formulations that act as nanocarriers of active ingredients. In this work, the droplet size distribution, rheology and physical stability of nanoemulsions with improved properties containing rosemary essential oil and biopolymers as a function of the concentration of these polysaccharides was investigated.

RESULTS: Mean diameters below 150 nm were achieved, indicating nanostructures were obtained. Regardless of gum type, a gel-like structure and a shear thinning behaviour was achieved. In addition, an increase of G', G" and viscosity and a decrease of J_0, J_1, J_2, λ_1 and λ_2 with increasing gum concentration were observed, due to the formation of a three-dimensional network in the aqueous phase. Slight differences between nanoemulsions containing welan or xanthan were found. Creaming, depletion flocculation and gel aggregation were the main destabilization processes at low, intermediate and high gum concentration, respectively. 0.4 wt% gum nanoemulsion exhibited the best physical stability.

CONCLUSION: These stable and sustainable nanoemulsions with improved rheological properties contribute to the development of biodegradable and non-toxic food or

agrochemical products.

Keywords: Nano**e**mulsion, rosemary essential oil, biopolymer, rheology, physical stability.

1. INTRODUCTION

In recent years, numerous studies about nanoemulsions have been developed due to the interest of these formulations as delivery systems for lipophilic active compounds. However, nanoemulsions are Newtonian and lack the desired rheological properties for some applications. Thus, for example in the case of an emulsion-based agrochemical or food product, this product should possess a non-Newtonian behaviour with high viscosity at rest in order to prevent its destabilization during lifetime, but at the same time, with low viscosity under an applied stress in order to facilitate the handling. In addition, a gel-like structure is also interesting due to the fact that is correlated with a higher stability of emulsions against creaming. This rheological behaviour can be obtained with these structured nanoemulsions which can be used not only in agrochemical or food applications but also in pharmaceutic, medical or cosmetic applications. Currently, another aspect to consider when formulating a product is the growing interest of consumers in the use of natural products and sustainable raw materials.

Natural polymers, actually strongly demanded due not only to their thickening, stabilizing or emulsifying properties, but also their biocompatibility, biodegradability and non-toxicity, can be used to enhance the rheological properties and improve the shelf life of nanoemulsions. Specifically, xanthan gum advanced performance and welan gum were used in this study.

Xanthan gum (XG) is a natural high molecular weight anionic polysaccharide produced by aerobic fermentation of sugars by the microorganism *Xanthomonas campestris*.[1] There are numerous products in different fields which use xanthan gum. Cleaners, coatings, polishes and products in the agricultural and food industry also use xanthan gum.[2] Specifically, advanced performance food grade xanthan gum is suitable for sauces, milk and cream products or beverages.

Welan gum is an anionic extracellular polysaccharide produced by the micro-organism *Sphingomonas sp.*, namely from *Alcaligenes sp.,* ATCC 31555.[3] Although welan gum is rarely used in emulsions, in the last few years, there have been a number of studies concerning its use in this type of formulations. For example, welan gum may be used as an ingredient in food products in which it can act as a thickening, suspending, binding, emulsifying, stabilizing and viscosifying agent.[3]

The high antioxidant and antibacterial activity of REO against L. monoctytogenes bacteria and also against *Escherichia coli*, *Salmonella indiana* and *Listeria innocua* has been attributed to the presence of its main components such as α- pinene, 1,8-cineole,

camphor, myrcene, camphene, borneol and verbenone.[4–6] REO is used in a wide range of applications, such as in food, in cosmetics, in nutraceuticals, agrochemical, phytomedicines and aromatherapeutical products.[7,8]

In this study, a non-ionic surfactant known as Appyclean 6548 was used. This surfactant belongs to the alkyl polypentoside (APP) group. Appyclean is a family of environmentally friendly surfactants possessing an Ecocert certificate, which are very desirable for using in green formulations. Rosemary oil/water emulsions has been demonstrated to have high physical stability using Appycelan 6548 (D-pentose, oligomeric C14/18 alkylglycoside) as surfactant.[9] However, this system exhibits low viscosity, a Newtonian flow behaviour and lacks a linear viscoelastic region. In addition, this emulsion suffers a destabilization by creaming. It is to be expected that the addition of gum causes the formation of a network capable of delaying the creaming and providing viscoelasticity to the nanoemulsion. Thus, the development of sustainable nanoemulsions with desirable rheological characteristic could be achieved by varying the concentration of gum. Therefore, the main objective of this work was to study the influence of the concentration of two different polysaccharides (welan and xanthan advanced performance gum) on the droplet size distribution, rheology and physical stability of nanoemulsions formulated with REO and Appyclean 6548, a non-ionic and renewable bio-based surfactant. These stable and sustainable nanoemulsions with improved rheological properties can be useful to the food and agrochemical industries as food preservative or insecticide due to the antimicrobial properties of the rosemary oil.[10,11] Furthermore, they can be useful to develop lipophilic active-loaded products with novel applications.

2. MATERIALS AND METHODS.

Materials.

All components of nanoemulsions are derived from natural sources. Thus, the surfactant is wheat-derived, while the dispersed phase is rosemary essential oil (REO) which is distilled from Rosemarinus officianalis L. REO was supplied by Sigma Aldrich (Spain) and Appyclean 6548 was purchased from Wheatoleo (France). Additionally, xanthan gum (KELTROL® Advanced Performance) of molecular weight about $2 \cdot 10^6$ /mol and welan gum (K1A96) of molecular weight about $6.6 \cdot 10^5$ g/mol were used as stabilizing

biopolymers. Both gums were kindly provided by CP Kelco Company (San Diego, USA). Ultrapure water was used to prepare the aqueous phase.

Emulsification Procedure.

The aqueous phases were prepared by dissolving 0.1/100 g sodium azide in ultrapure water cleansed using a Milli-Q water purification system. The dispersed phases were formed by the emulsifier, Appyclean 6548 (4/100 g), and rosemary oil (20/100 g). Emulsions were prepared following the protocol reported by Martin-Piñero.[9] Finally, in order to obtain the structured nanoemulsion, the gum was added very slowly into the final emulsions at room temperature. The emulsions were mechanically stirred using an Ultra-turrax device (IKA Janke and kunkel, IKA-Labortechnik) for two hours up to a maximum of 520 x g (800rpm). A total of 11 samples were prepared using different kinds of biopolymer (xanthan and welan gums) at different concentrations (0, 0.1/100, 0.2/100, 0.3/100, 0.4/100, 0.5/100 g).

Droplet size distribution (DSD) measurements.

Size distribution of oil droplets was determined by the laser diffraction technique (Mastersizer 2000, Malvern, Worcestershire, United Kingdom). Aging time evolution of the volume-weighted mean diameter ($D_{4,3}$) of the emulsion droplets and the span values were studied for 28 days. $D_{4,3}$ values were obtained from the following equation:

$$D_{4,3} = \frac{\sum_{i=1}^{N} n_i d_i^4}{\sum_{i=1}^{N} n_i d_i^3} \qquad \text{(Eq. 1)}$$

where n_i is the number of droplets with diameter d_i. On the other hand, span values are given by:

$$Span = \frac{D(v,0.9) - D(v,0.1)}{D(v,0.5)} \qquad \text{(Eq. 2)}$$

where D(ν, 0.9), D(ν, 0.5) and D(ν, 0.1) are the highest droplet size contained in 90%, 50% and 10% of volume of dispersed phase, respectively.

Physical Stability.

The physical stability of rosemary essential oil nanoemulsions was evaluated for 28 days at 30ºC by a Turbiscan Lab Expert device (Formulaction, Toulouse, France) using the multiple light scattering technique. Emulsion stability was studied by analysing the variation of the backscattering (BS) profiles as a function of time. The intensity of the

scattered light makes it possible to determine the major mechanism of destabilization in each sample as well as the kinetics of the destabilization process.

Rheology.

Flow curves were performed with a Modular Advanced Rheometer System (Haake MARS, Thermo Electron Corp., Germany) and a double cone geometry (60/1º) for nanoemulsions with gum concentration lower than 0.3/100 g. Nanoemulsions with 0.4/100 g and 0.5/100 g of gums were measured using a serrated plate-plate sensor (60 mm). Flow curves were carried out in a range of 0.05 to 20 Pa.

Frequency sweeps were obtained from 0.05 to 15 rad/s at fixed shear stress within the linear viscoelastic region. Creep experiments were performed for constant shear stresses (0.05 Pa) for 3600 s.

Equilibration time prior to rheological tests was 300s. All measurements were performed at 20 °C ± 0.1 °C using a C5P Phoenix circulator (Thermo-Scientific, Germany).

All measurements were carried out in triplicate and the values shown are the average of the three replicates. The standard deviation of all measurements was lower than 5-10% for all the samples.

Statical analysis.

A general linear model – one-way ANOVA – was used to determine significant differences ($P \leq 0.05$) between samples, followed by the post-hoc Tukey HSD test. All statistical treatments were done with the software Infostat, version 2017 (Facultad de Ciencias Agropecuarias, Universidad Nacional de Cordoba, Cordoba, Argentina).

3. RESULTS AND DISCUSSION.

3.1.Droplet Size Distribution

Figure 1 shows as inset, by way of example, the droplet size distribution of the emulsions containing 0.4/100 g WG and 0wt% gum as reference, both 24 hours after preparation. As can be observed, 0wt% gum emulsion is monomodal (span = 0.74±0.01) and the volumetric weight diameter is 149 ± 6 nm. By contrast, the addition of 0.4/100 g of biopolymer provokes a shift of DSD towards bigger droplets and the distribution was bimodal. In order to gain a deep insight into the results obtained, measurements of laser

diffraction at different conditions of dispersion (introducing ultrasounds) as a function of time in the measuring cell were carried out. Results obtained are shown in Figure 1. As can be observed, the droplet size distribution shifted towards smaller droplet sizes and was monomodal. These facts revealed that the emulsion was initially flocculated and proved that the second peak corresponded to air bubbles, which were removed. Therefore, ultrasonics break-up the agglomerates, making it possible to obtain the droplet size of the primary droplets. It should be noted that the addition of gum did not significantly change the DSD compared to the emulsion without gum. However, these results demonstrate the ability of both xanthan gum and welan gum to promote flocculation.

Figure 1

The influence of gum concentration and ageing time (1 and 28 days) on the values of $D_{4,3}$ and span of nanoemulsions are shown in Table 1. These parameters were selected because they were very sensitive to the aggregation of droplets and, therefore, to detect destabilization by coalescence or Ostwald ripening. As can be observed, nanoemulsions containing welan gum exhibited similar volumetric mean diameter 24 hours after preparation, except for the one formulated with 0.4/100 g, which presented the highest values of both $D_{4,3}$ and span. Similar results were found for nanoemulsions containing xanthan gum but it was the nanoemulsion with 0.5/100 g of gum which exhibited the highest values of both parameters. Ageing time has an influence on distribution parameters for nanoemulsions containing 0.3/100 g and 0.5/100 g of WG and for nanoemulsions with 0.3/100 g of APXG. For these nanoemulsions, an increase in volumetric mean diameter and span occurred with time, suggesting the occurrence of a destabilization process by coalescence.[12,13] It is noteworthy that the increase in droplet size observed is not caused by flocculation due to the fact that the aggregates were broken up before the measurement was carried out, as mentioned above.

3.2. SAOS tests

Regardless of type of gum used, all nanoemulsions exhibited a measurable linear viscoelastic range except the one with 0.1/100 g of biopolymer. For this reason, results at this concentration of gum are not shown. The effects of gum concentration on storage, G‘, and loss, G”, moduli as a function of frequency for microfluidized rosemary O/W emulsions, aged for 24 hours, are shown in Figure 2. All nanoemulsions showed that G’

was larger than G" over the whole measured frequency range, indicating that the elastic component was predominant in the viscoelasticity. Furthermore, a slight frequency dependence of both moduli was found. These profiles are consistent with a weak gel-like viscoelastic structure and they indicate more semi-solid-like than liquid-like behaviour.

As expected, G´ and G´´ increased with gum concentration, consistent with a tri-dimensional macromolecular network with more elastic and viscous behaviour. As occurs in gum solutions with gel-like structure, both moduli have an exponential relationship with the gum concentration which was fitted to the following equations:

$$\log G' = loga + b * logC \quad \text{(Eq. 3)}$$

$$\log G'' = loga' + b' * logC \quad \text{(Eq. 4)}$$

where b and b' are the power law index of G' and G", respectively, and a and a' are elastic coefficient and viscous coefficient, respectively. As can be observed in Table 2, a and a' values were higher for nanoemulsions containing welan gum, indicating that these nanoemulsions are more elastic and viscous than nanoemulsions containing xanthan gum. Likewise, nanoemulsions with welan gum were more dependent on the concentration, as can be deduced from the higher values of the b and b' parameters.

Figure 2

Table 2

From the frequency sweeps, values for loss tangent were calculated. tan δ is defined as the ratio of loss modulus and storage modulus, which is used to detect differences in the temporary structure of samples.

$$tan\delta = \frac{G''}{G'} \qquad \text{(Eq. 5)}$$

When the loss tangent approaches zero, the elastic structure of the system predominates, whereas if it exceeds 1 value the system is considered viscous. As shown in Figure 3A, the elastic component predominates over the viscous one over the whole frequency range with 0.23< tanδ <0.33 and 0.29< tanδ <0.68 for nanoemulsions with APXG and WG, respectively. The tanδ values were lower than 1, indicating that the molecular network was strong and permanent.[14] In all cases, $0.1 < tan\delta < 1$ indicating that these nanoemulsions do not behave as true gels, but as weak gels such as discussed above.

All nanoemulsions containing gum as thickener showed a clear minimum in tan δ value except for the emulsion with 0.2/100 g WG. From this minimum it is possible to calculate the plateau modulus, G_N^0: [15]

$$G_N^0 = [G']_{\tan\partial \to \min} \quad \text{(Eq. 6)}$$

The higher the G_N^0 value, the higher the entanglement density between polymeric molecules.[16] As expected (Figure 3B), an increase in gum concentration caused an increase in the plateau modulus value, indicating an increase in the entanglement density. It should be noted that the values of plateau modulus are slightly higher in nanoemulsions containing welan gum than those containing xanthan gum.

All results are consistent with a double helix conformation for both gums. However, it seems strange that welan gum, despite having lower molecular weight than xanthan, provides nanoemulsions with greater viscoelasticity. This result could be explained by the fact that welan gum adopts a different distribution pattern.[17]

Figure 3

The evolution with ageing time of G′ values at an intermediate frequency within the frequency range studied (6.8 rad/s) for all nanoemulsions is illustrated in Figure 4. Fig. 4 shows that for the first days nanoemulsions exhibited a slight tendency to a decrease in G′, made more marked by increasing the concentration of gum in the emulsion. Subsequently, the values remained constant. A decrease in G' with ageing time could be attributed to an increase in droplet size, either by coalescence or by flocculation, which provokes a decrease in the area of contact between droplets, weakening the interactions between them and, therefore, a decrease in elastic properties. All nanoemulsions exhibited values of G' above G" after 27 days, indicating that the elastic behaviour was preserved. In addition, the nanoemulsions containing welan gum, similarly that happens at 24 h, exhibited higher G' values than those ones formulated with xanthan gum. Note that the results at ageing time higher than 14 days for 0.2/100 g welan gum are not shown due to the breakdown of the emulsion.

Figure 4

3.3.Creep tests

Figure 5 shows creep test results in terms of shear creep compliance versus test time at constant shear stress (0.05 Pa) as a function of WG and APXG concentration added into the emulsions. All samples showed a typical response of weak viscoelastic solids with a non-linear response to strain. As expected, the strongest nanoemulsion, which was the lowest line, represented the nanoemulsion with 0.5/100 g of concentration of gum added. The strength of the other samples can be deduced from the level of compliance exhibited in Figure 5, namely nanoemulsions with 0.4/100 g gum > 0.3/100 g gum.

In order to achieve a better analysis of the results, the creep curves were fitted to the Burger model, which consists of Maxwell and Kelvin elements connected in series. In this particular case, two elements were used.

$$J(t) = J_0 + \sum_{i=1}^{2} J_i \left(1 - e^{\frac{-t}{\tau 1}}\right) + \frac{t}{y_N} \qquad \text{Eq. (7)}$$

were J is the compliance as a function of time, t, $J_0 = 1/G_0$ (1/Pa) is the initial instantaneous compliance, $J_1 = 1/G_1$ and $J_2 = 1/G_2$ are retarded compliances for the Kelvin model (1/Pa), $\tau 1$ and $\tau 2$ are the retardation time, which is the time needed to reach maximum deformation for the Kelvin model (s), t is time (s) and η_N is the Newtonian viscosity (Pa·s) in the steady state.

Figure 5

All parameters derived from the model are listed in Table 3. The nanoemulsions with 0.5/100 g of gum, regardless of the type of gum, showed the lowest values of J_0, J_1, J_2, λ_1 and λ_2 but the highest value of η_0. In contrast, the highest values of of J_0, J_1, J_2, λ_1 and λ_2 but the lowest value of η_0 was exhibited by the nanoemulsion containing the lowest gum concentration. These results mean that the structure of nanoemulsions increases their viscoelastic properties by increasing their gum content due to the fact that they increase their strength to flow, being more difficult to deform under the imposed stress. From an overall point of view, no significant differences were observed between the creep response of nanoemulsions with WG or with APXG.

Table 3

3.4.Steady state flow tests

The flow behaviour of nanoemulsions studied is illustrated, by way of example, for nanoemulsions containing APXG as a function of their concentration, in Figure 7. It is

important to note that emulsion without gum exhibited Newtonian flow behaviour. This Newtonian behaviour has been previously reported by Martín-Piñero et al. ($\mu = 3.19 \pm 0.02$mPas).[9] As shown in Figure 6, the addition of gum changes the flow behaviour. Thus, all nanoemulsions presented a shear-thinning behaviour characterized by a decrease in viscosity as the shear rate increased, which is related to the orientation of macromolecules and droplets in the direction of flow. Furthermore, at low shear rates a Newtonian plateau can also be observed up to a critical shear rate. This region is better observed at higher gum concentrations (0.3, 0.4 and 0.5/100 g). The viscosity of this region is so-called zero-shear viscosity (η_0), which is related to the stability of nanoemulsions against creaming.[18] In addition, McClements also indicated that at low shear rates the hydrodynamic forces are not strong enough to break up the flocs, which then act like particles with fixed size and shape, resulting in a constant viscosity. The apparent viscosity of nanoemulsions increased with increasing gum concentration. Obviously, the entanglements of chains of the macromolecules increased with increasing the concentration, which caused the corresponding increase in viscosity.

Different models can be used to model the flow behaviour of Non-Newtonian systems, such as Herschel-Bulkley, power law, etc. Nevertheless, for shear thinning fluids, the Cross model is appropriate since it is able to describe this behaviour quite well and in a wide range of shear rates.

$$\eta = \frac{\eta_0}{1+(\frac{\gamma}{\gamma_c})^{1-n}} \quad \text{(Eq. 8)}$$

where γ_c is related to the critical shear rate for the onset of the shear-thinning response, η_0 stands for the zero-shear viscosity and (1-n) is a parameter related to the slope of the power-law region; n being the so-called "flow index". For shear thinning materials, $0 < n < 1$. All nanoemulsions exhibited a good fit to the Cross equation ($R^2 = 0.999$).

Zero-shear viscosity values (η_0) and the flow index (n) at 1 and 28 days are shown in Table 4 as a function of the gum type and concentration. Nanoemulsions with welan gum aged for 24 hours exhibited a tendency to a decrease in the critical shear rate with increasing concentration of gum. On the other hand, nanoemulsions with xanthan only show this decrease when increasing the concentration from 0.1 to 0.3/100 g. From this value, it is constant. Except for 0.1/100 g of gum, all nanoemulsions showed a Newtonian region lower than 0.01 s^{-1}, indicating that the emulsion keeps its shear thinning behaviour

at low shear rates. The aging time causes an increase in the critical values of the shear rate for the beginning of the shear thinning behaviour.

Regarding zero-shear viscosity, at 24 hours of storage time, nanoemulsions with the lowest gum concentration (0.1/100 g) showed the lowest zero-shear viscosity. In contrast, those nanoemulsions containing the highest gum content exhibited the highest viscosity value. In general, by increasing the concentration of gum, an increase in viscosity occurred, irrespective of the nature of the gum. This result may be explained by the thickener effect of biopolymers in the bulk phase.[19]

At low gum concentration, nanoemulsions with xanthan gum are more viscous than those ones containing welan gum. However, at the highest concentration studied the opposite occurred. Furthermore, nanoemulsions with welan gum turned out to be more dependent on the concentration than those containing xanthan gum, although not significantly. It is noteworthy that the addition of gums into the emulsions to obtain the nanoemulsions not only changes the flow behaviour, but it also considerably increases their viscosity. This augmentation is very significant at the highest gum concentrations (for example, 3.9 mPa·s compared to 3000 Pa·s).

Except for emulsion with 0.2/100 g of welan gum, zero-shear viscosity decreased for all nanoemulsions after 28 days of study. A decrease in η_0 with aging time could be related to a destabilization of the system by coalescence or flocculation.

On the other hand, the low values of the flow index indicated that these nanoemulsions exhibited a strong shear thinning behaviour. The nanoemulsions with lower gum concentration were less shear thinning. In contrast, the nanoemulsions with stronger shear thinning properties were those containing the highest gum concentration. It should be noted that nanoemulsions formulated with xanthan gum are slightly more pseudoplastic than nanoemulsions containing welan gum. The flow indexes slightly increase with ageing time but not significantly.

In summary, as expected, η_0 increases and n decreases as a function of gum concentration. On the other hand, ageing time provokes a decrease in viscosity and a decrease in non-Newtonian behaviour.

Figure 6

Table 4

3.5. Multiple light scattering results

Profiles of backscattering as a function of the height of the measuring cell and as a function of ageing time for nanoemulsions formulated with xanthan or welan gum at different concentrations of gum are illustrated in Figure 7.

Figure 7

Different destabilization processes have been found as a function of gum concentration in nanoemulsions. Creaming was the main destabilization process for nanoemulsions with the lowest amount of gum (0.1/100 g for APXG and 0.1/100 g and 0.2/100 g for welan gum). The density difference between continuous and disperse phases besides the low viscosity of these systems favoured gravitational separation. Finally, a layer of serum and a layer of cream were observed. At intermediate gum concentrations (0.2/100 g and 0.3/100 g for APXG and 0.3/100 g for welan gum) an increase of droplet size was observed, which can be attributed to a depletion flocculation mechanism caused by the non-adsorbed polymer. This process, sometimes, led to coalescence due to the flocs rise and collide, breaking their interfaces. Depletion flocculation process happened at a higher concentration of gum when welan was used due to its lower molecular weight.[20] At a higher gum level (0.4/100 g), nanoemulsions stable against creaming and flocculation were obtained due to the formation of three-dimensional viscoelastic network able to immobilize the droplets. Finally, at the highest gum concentration studied (0.5/100 g) an increase in droplet size (an increase in BS profile over the entire tube length) was observed which was slight for nanoemulsions containing APXG but significant for ones containing welan gum. For latter a destabilization by coalescence occurred according to the results obtained by laser diffraction. We can hypothesize that this concentration of gum is high enough for gel-like aggregates to form, which facilitate the flocculation of droplets and a further coalescence. All results obtained by multiple light scattering were consistent with those obtained by laser diffraction and rheology.

Since the destabilization mechanisms of these nanoemulsions are creaming and an increase in the size of droplets (flocculation or/and coalescence), the turbiscan stability index (TSI) was calculated from equation 10 as a global destabilization parameter. This parameter was previously used by other authors:[21]

$$TSI = \sum_i \frac{\sum_h |scan_i(h) - scan_{i-1}(h)|}{H} \qquad \text{(Eq. 9)}$$

where: $scan_i(h)$ is the average backscattering for each time (i) of measurement, $scan_{i-1}(h)$ is the average backscattering for the i – 1 time of measurement and H is the number of scans for each sample. Thus, a high value of TSI corresponds to a less stable system. The TSI global values for all nanoemulsions studied are shown in Figure 8. It can be observed that TSI was lower (higher stability) for those samples formulated with 0.4wt% of gum, regardless of the gum type. These two nanoemulsions showed TSI values after 28 days of store time of 3.80 and 3.85 for APXG and WG, respectively. Nanoemulsions formulated with lower gum concentration showed the highest values of TSI and a very marked slope at short ageing time, indicating not only their lower stability but also their fast destabilization. Nanoemulsions with 0.5wt% of APXG showed a similar behaviour to 0.4wt%.

Figure 8

Conclusions.

In this paper, nanoemulsions of rosemary essential oil and biopolymers (advanced performance xanthan or welan gum) were obtained. Firstly, emulsions with rosemary essential oil and a wheat-waste derived surfactant were obtained by microfluidization. In a further step, welan or advanced performance xanthan gum were added. The influence of the gum concentration on the droplet size distribution, rheological properties and physical stability of emulsions was investigated. A gel-like structure and a shear thinning flow behaviour were found. An increase in the gum concentration provoked an augmentation of both the viscoelastic moduli and viscosity and a decrease in J_0, J_1, J_2, λ_1 and λ_2 due to the formation of a three-dimensional network in the aqueous phase due to the presence of biopolymers. Emulsions containing welan gum were more elastic and viscous than those containing APXG but, at the same time, they were more dependent on the concentration. Regarding the droplet sizes, nanoemulsions were obtained. $D_{4,3}$ remained stable over time except for nanoemulsions containing 0.3/100 g of xanthan gum and those formulated with 0.3/100 g and 0.5/100 g of welan gum, which exhibited coalescence. Different destabilization mechanisms were found, depending on the gum concentration. Creaming was the main destabilization process at low gum level and depletion flocculation, sometimes with coalescence, at intermediate gum level. At 0.4/100 g of gum, stable nanoemulsions were obtained and, finally, at 0.5/100 g of gum a new trend to destabilization was observed, which was significant for nanoemulsions

containing welan gum, probably due to the formation of gel-like aggregates. Slight differences were observed between nanoemulsions with xanthan or welan which could be explained by the differences in viscosity and molecular weight of both polysaccharides. This work contributes to attempts to meet the demand from consumers for more biodegradable, biocompatible and non-toxic food or agrochemical products..

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Conflicts of interest

The authors declare no conflict of interest.

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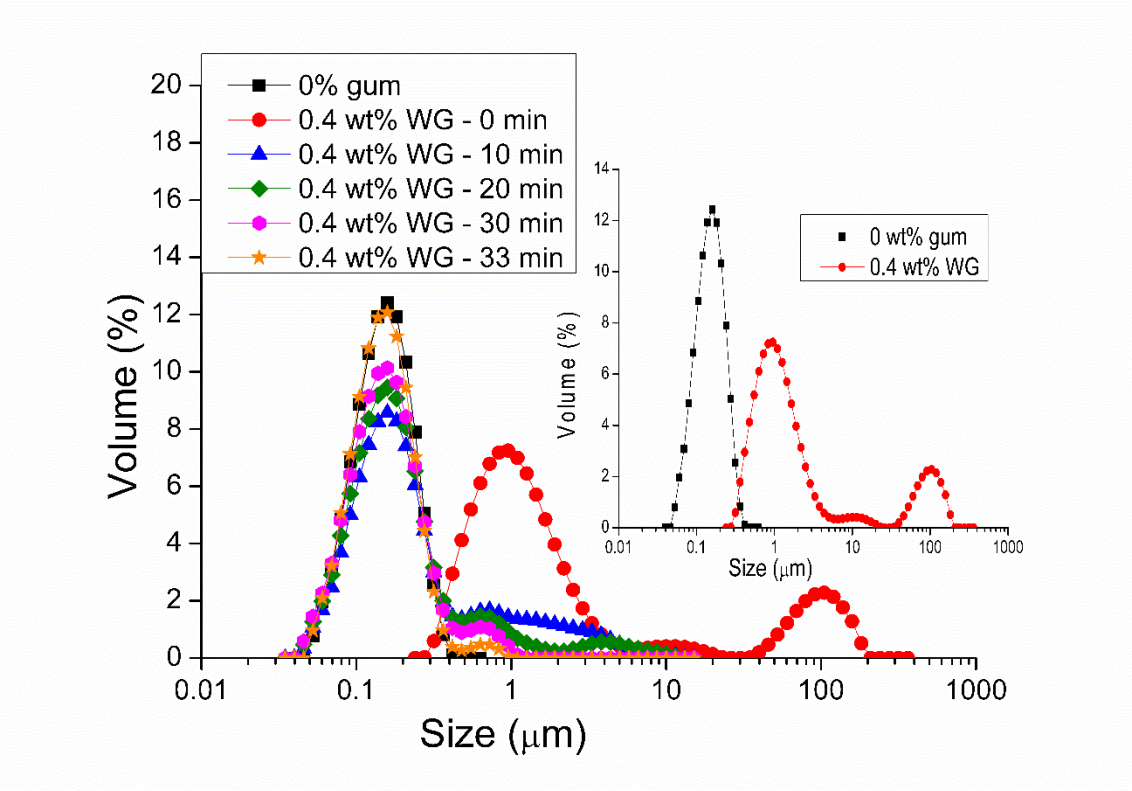
Figures



488488

489 Figure 1. Droplet size distribution for emulsions containing 0.4wt% of WG as function of
490 ultrasound time in the measurement cell and as inset, 0.4 wt% WG without ultrasound and 0
491 wt% gum

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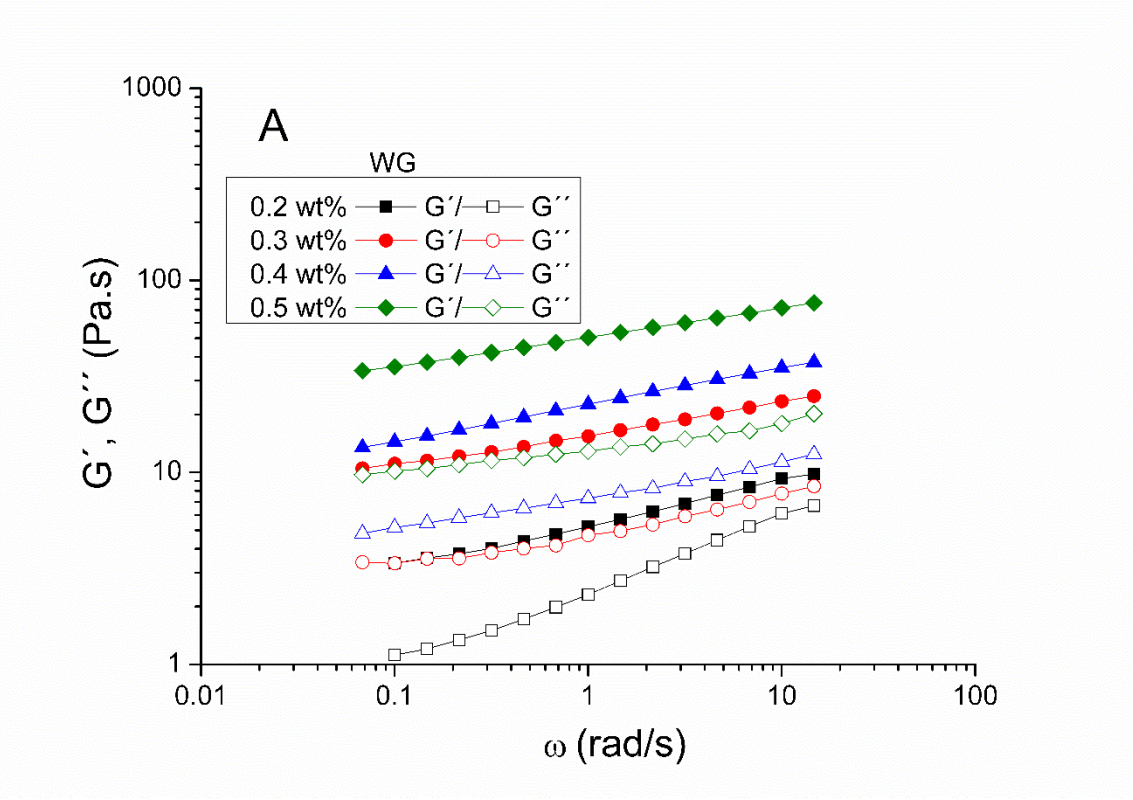

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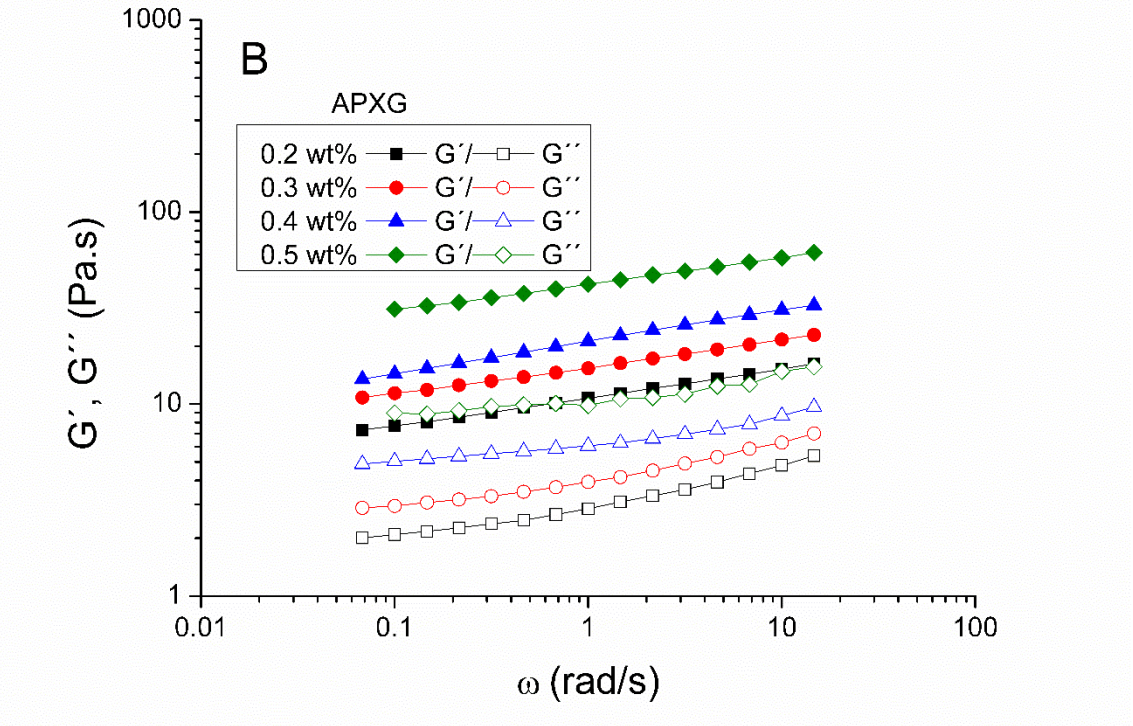
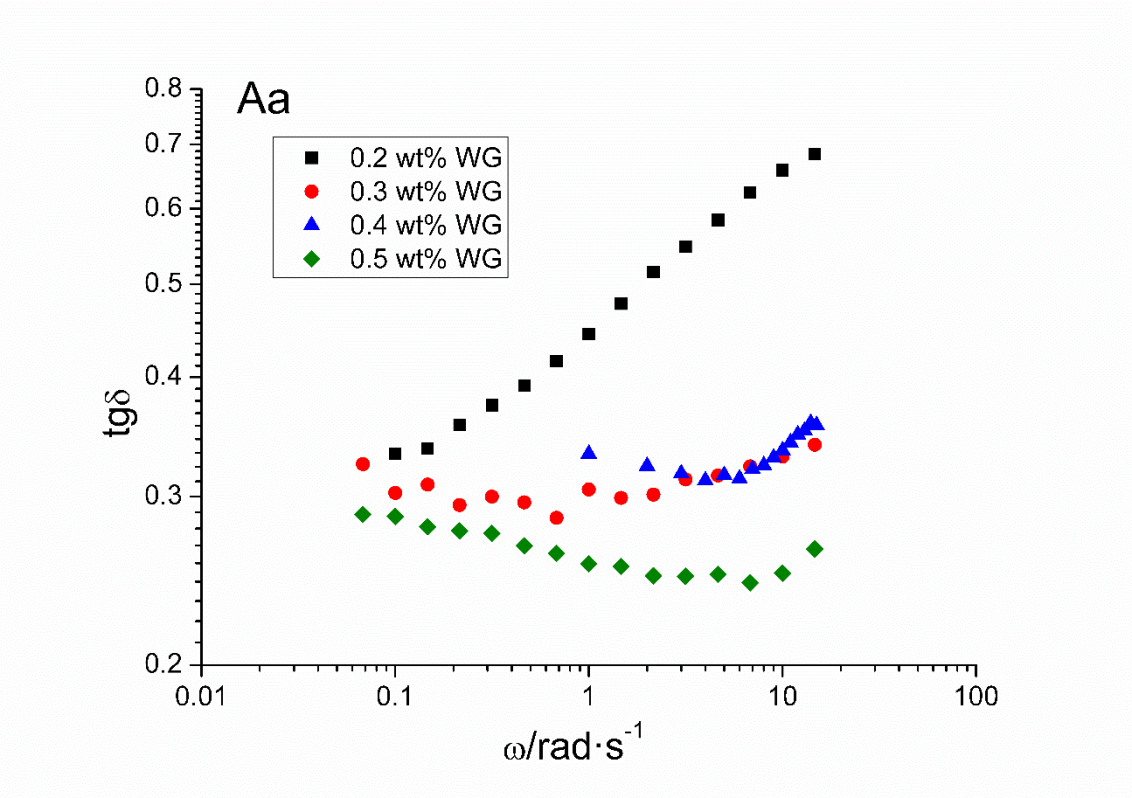
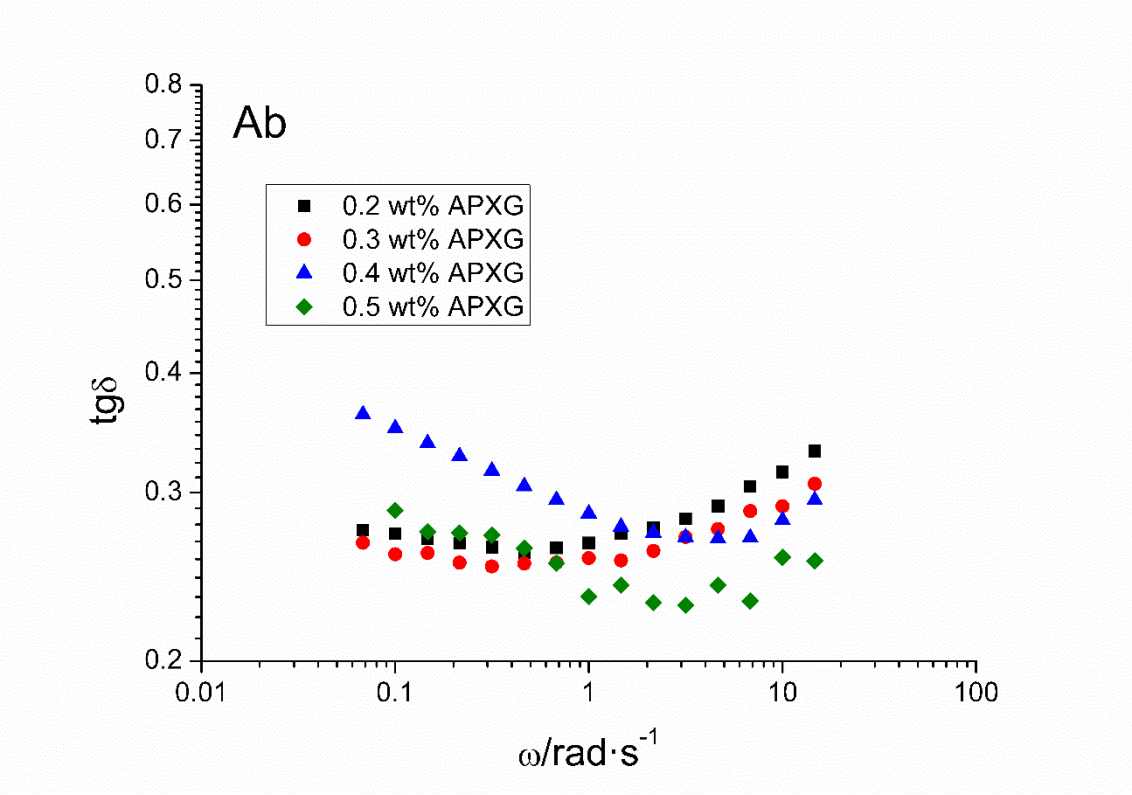


495

496 Figure 2. Influence of gum concentration on the mechanical spectra for nanoemulsions
497 containing a)WG or b) APXG. Temperature = 20 °C.


Aa
0.2 wt% WG
0.3 wt% WG
0.4 wt% WG
0.5 wt% WG
tgδ
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.01
0.1
1
10
100
ω/rad·s-1

498


Ab
0.2 wt% APXG
0.3 wt% APXG
0.4 wt% APXG
0.5 wt% APXG
tgδ
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.01
0.1
1
10
100
ω/rad·s-1

499

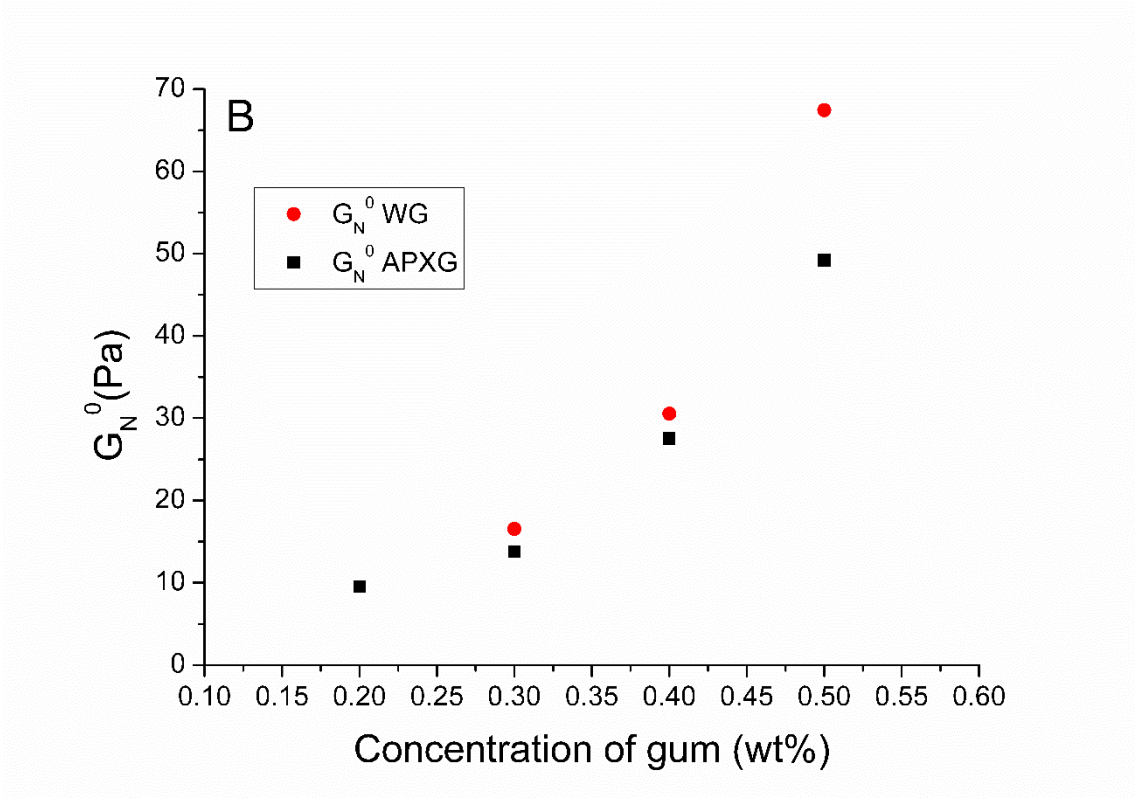


Figure 3. A) Influence of frequency on loss tangent of rosemary essential oil nanoemulsions 1-day aged containing a) WG or b) APXG as a function of the gum concentration. B) Plateau modulus of rosemary essential oil nanoemulsions versus gum concentration as a function of the type of gum.

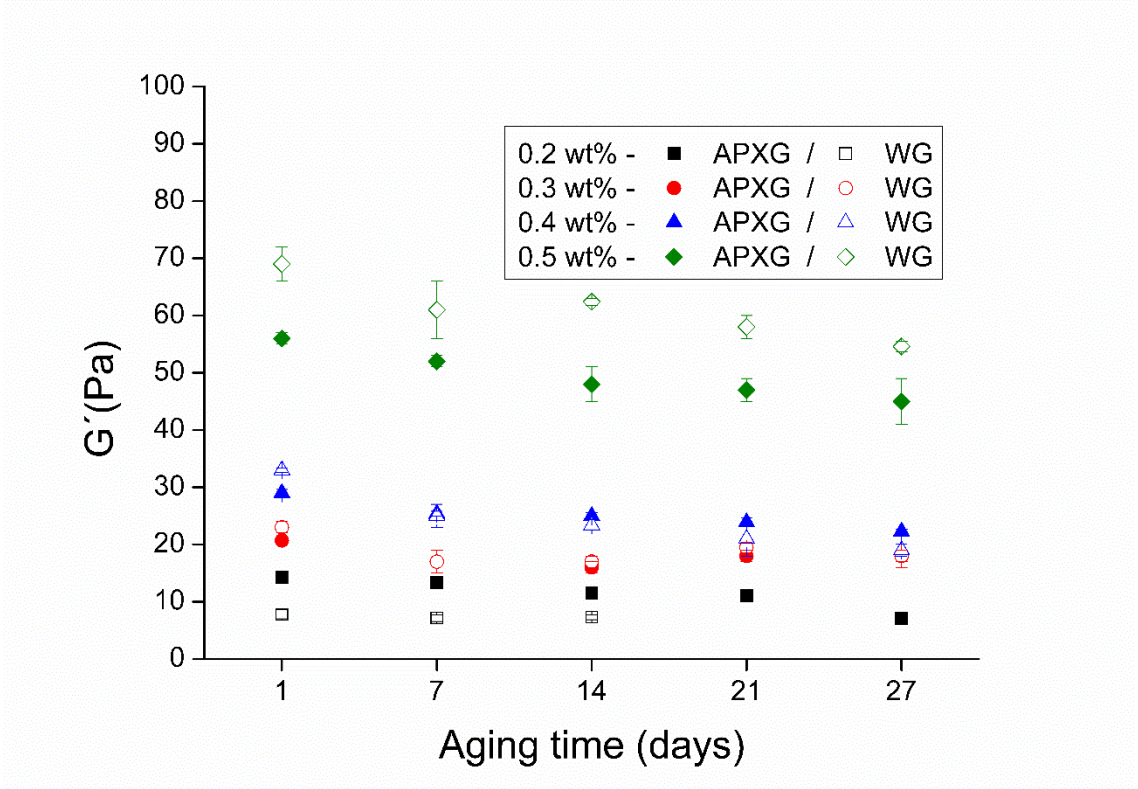


Figure 4. Influence of aging time on the elastic modulus at 6.8 rad/s of rosemary essential oil nanoemulsions containing WG or APXG as a function of gum concentration. Temperature = 20 °C.

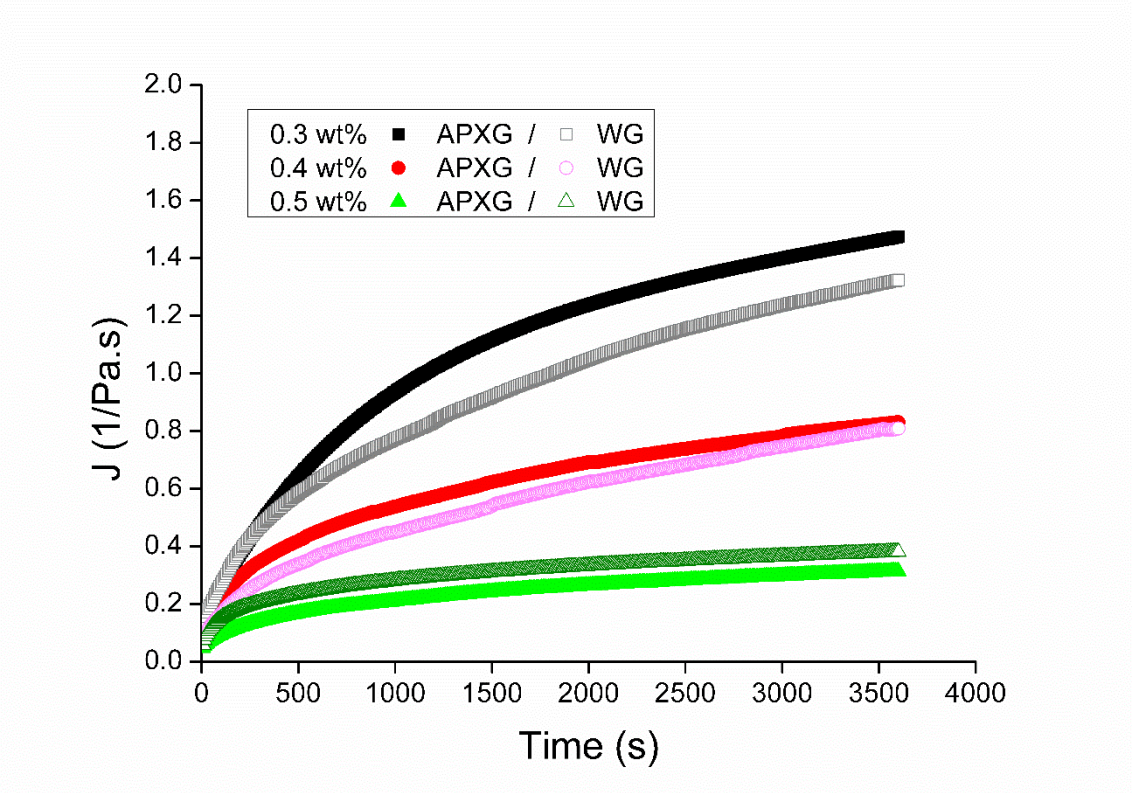
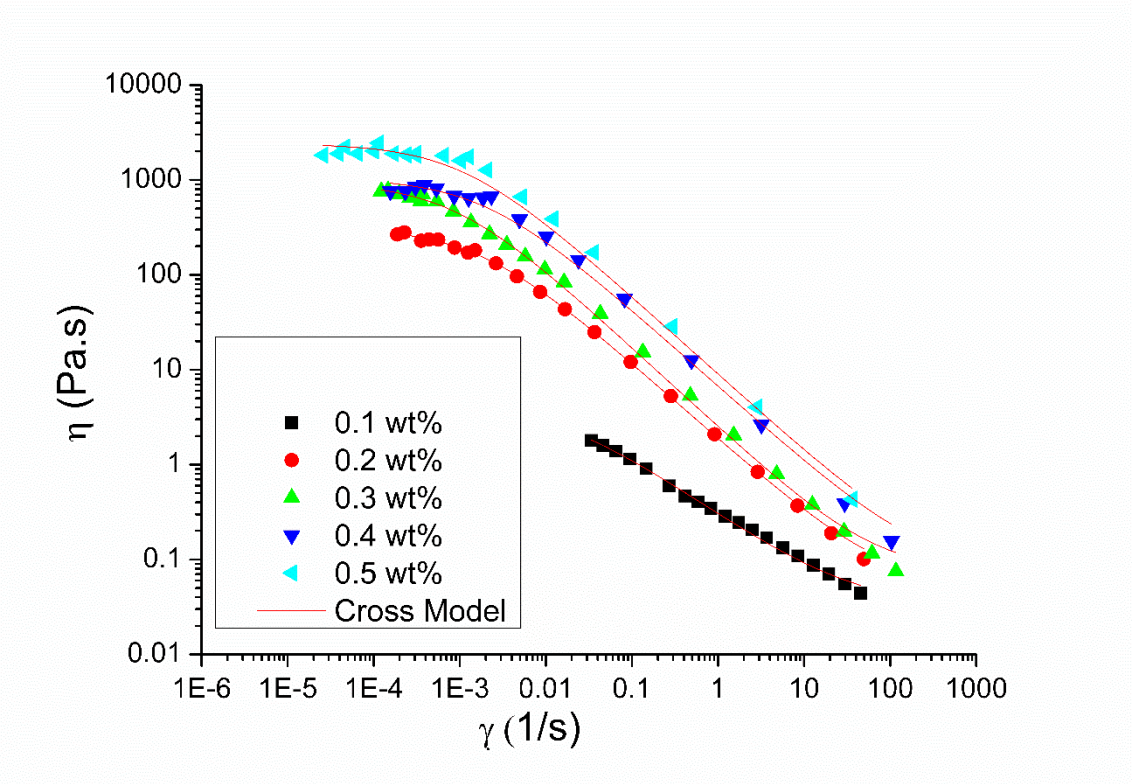


Figure 5. Creep compliance versus time of rosemary essential oil nanoemulsions containing WG or APXG as a function of the gum concentration. Shear stress= 0.05 Pa. Temperature 20 °C.



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517 Figure 6. Flow curves of rosemary essential oil nanoemulsions containing different
518 concentrations of APXG. Continuous lines correspond to the Cross model fitting equation.
519 Temperature = 20 °C.

520

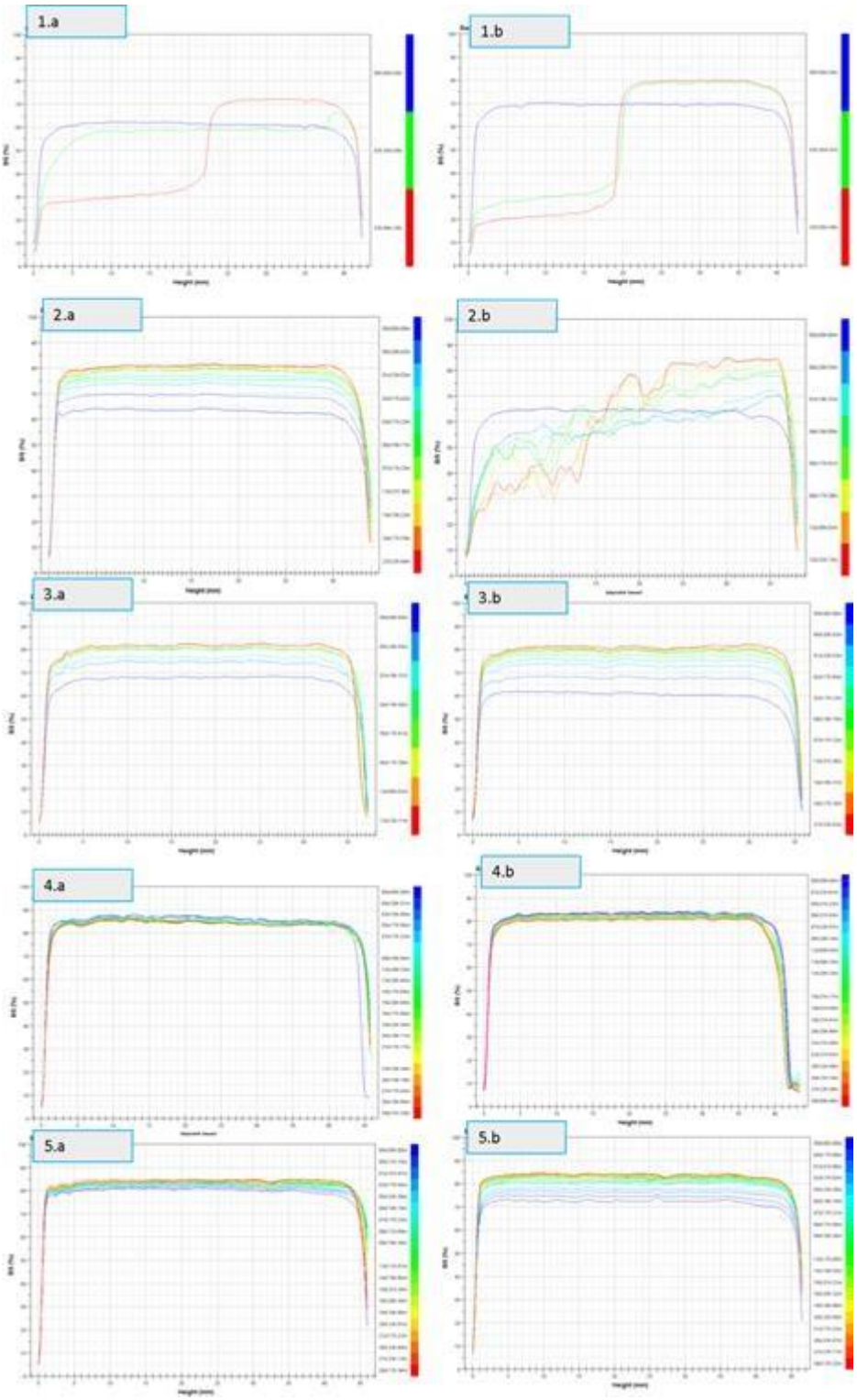
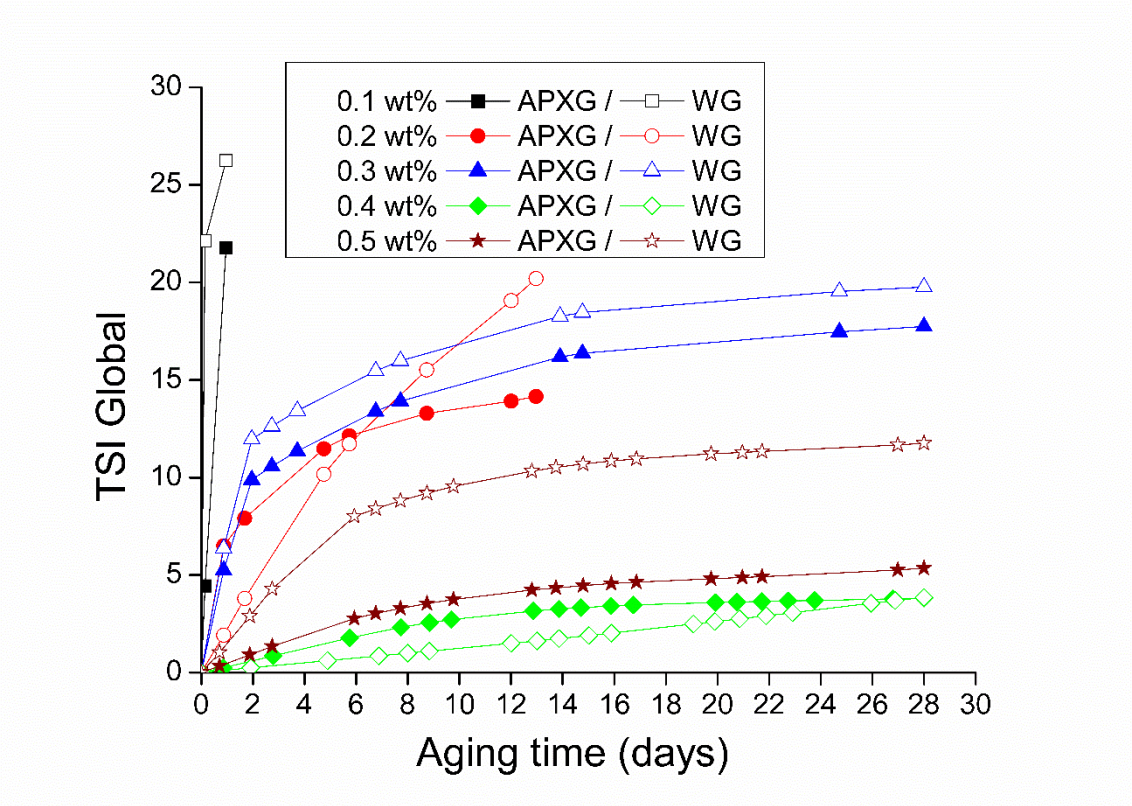


Figure 7. BS profiles versus measuring cell height of rosemary essential oil nanoemulsions containing a) APXG and b) WG at different concentrations of gum (1 (0.1/100g), 2 (0.2/100g), 3 (0.3/100 g), 4 (0.4/100 g) and 5 (0.5/100 g)) as a function of aging time.



526

527 Figure 8. Turbiscan stability index versus aging time of rosemary essential oil nanoemulsions
528 containing WG (closed symbols) or APXG (open symbols) as a function of the gum concentration.
529 Temperature = 20^{0}C

530

531 **Tables**

532 Table 1. Volume mean diameter and span at 1 and 28 days of Rosemary essential oil
533 nanoemulsions containing WG or APXG. Mean value ± standard deviation values by a
534 different superscripts within raw were significantly different (P<0.05).

Gum concentration (wt%)	Gum	$D_{4,3}$ - Day 1 (nm)	$D_{4,3}$ - Day 28 (nm)	Span (Day 1)	Span (Day 28)
0.1	APXG	147±1^a	-	1.1±0.4^a	-
	WG	148±1^a	-	1.09±0.02^{a,b}	-
0.2	APXG	149±5^a	150±4^a	0.86±0.08^{a,b}	1.0±0.1^{a,b}
	WG	145±2^a	146±1^a	0.771±0.01^a	0.744±0.03^a
0.3	APXG	154±2^a	204±12^a	0.8±0.1^a	1.7±0.1^b

	WG	155±1[a]	179±3[a]	0.743±0.01[a]	1.4±0.03[a,b]
0.4	APXG	152±12[a]	154±15[a]	1.0±0.2[a,b]	1.0±0.2[a,b]
	WG	170±1[a]	177±10[a]	1.5±0.5[a,b]	1.4±0.2[a,b]
0.5	APXG	179±2[a]	180±1[a]	1.34±0.08[a,b]	1.3±0.1[a,b]
	WG	151±1[a]	200±3[a]	1.0±0.1[a,b]	2.7±0.1[c]

535

536

537 Table 2. Power law index of G' and G" at 1 rad/s (b', b") and elastic and viscous
538 coefficient (a', a"). Mean value ± standard deviation values by a different superscripts
539 within raw were significantly different (P<0.05).

Gum in nanoemulsion	a	SD_a	a'	$SD_{a'}$	b	SD_b	b'	$SD_{b'}$
WG	2.38[a]	0.12	1.63[a]	0.06	2.36[a]	0.24	1.83[a]	0.12
APXG	1.97[a]	0.16	1.34[a]	0.05	1.40[a]	0.31	1.33[a]	0.10

540

541 Table 3. Fitting parameters to the Burger model of creep tests results of rosemary
542 essential oil nanoemulsions 1 day aged containing WG or APXG. Mean value and
543 standard deviation values by a different superscripts within raw were significantly
544 different (P<0.05).

Concent. (wt%)	J_e^0 (1/Pa)		n_0 (Pa.s)		J_0 (1/Pa)		J_1 (1/Pa)		λ_1 (s)		J_2 (1/Pa)		λ_2 (s)	
Day 1	APXG	WG	APXG	WG	APXG	WG	APXG	WG	APXG	WG	APXG	WG	APXG	WG
0.2	1.3±0.5[a]		7158±300[a]		0.17±0.01[b]		0.038±0.01[a]		26.85±0.01[a]		1.1±0.5		803.2±0.1[b]	
0.3	0.8±0.5[a]	0.75±0.07[a]	9836±216[a]	7625±1090[a]	0.08±0.02[a,b]	0.119±0.001[a,b]	0.03±0.01[a]	0.04±0.01[a]	23.0±2[a]	125±21[c]	0.6±0.4	0.59±0.07	800±1[b]	897±11[c]
0.4	0.57±0.01[a]	0.6±0.2[a]	12698±1125[a,b]	8235±1284[a]	0.099±0.004[a,b]	0.10±0.03[a,b]	0.02±0.01[a]	0.039±0.001[a]	110±1[b,c]	130±7[c]	0.40±0.06	0.3±0.1	790±13[b]	780±9[b]
0.5	0.3±0.1[a]	0.22±0.09[a]	32840±5727[c]	31997±6872[b,c]	0.06±0.02[a]	0.05±0.01[a]	0.04±0.01[a]	0.039±0.001[a]	100±10[b,c]	66±5[a,b]	0.2±0.1	0.12±0.08	700±12[a]	662±12[a]

545

546 Table 4. Fitting parameters to the Cross model of flow curves of rosemary essential oil
547 nanoemulsions containing WG or APXG at 1 and 28 days of aging. Standar desviation: n_0
548 < 10%. n < 5% and Υ_c < 10%. Mean value by a different superscripts within raw were
549 significantly different (P<0.05).

Concent. (wt%)	n_0 (Pa.s)		$\Upsilon_c(s^{-1})$		n	
Day 1	WG	APXG	WG	APXG	WG	APXG
0.1	3.36^{a}	4.93^{a}	0.031^{b}	0.014^{a}	0.43^{h}	0.33^{g}
0.2	67.73^{a}	$316.45^{a,b}$	$1.6.10^{-3\ a}$	$1.7.\ 10^{-3\ a}$	0.24^{f}	$0.20^{e,f}$
0.3	$1000.50^{b,c,d}$	$934.31^{b,c,d}$	$6.6.\ 10^{-4\ a}$	$1.0.\ 10^{-3\ a}$	$0.18^{d,e}$	$0.16^{d,e}$
0.4	$1008.40^{b,c,d}$	$1042.73^{c,d}$	$4.8.\ 10^{-4\ a}$	$2.0.\ 10^{-3\ a}$	$0.14^{c,d}$	$0.10^{b,c}$
0.5	3313.0^{g}	2414.74^{f}	$4.0.\ 10^{-4\ a}$	$1.1.\ 10^{-3\ a}$	0.13^{g}	$0.07^{a,b}$

550

551

Day 28	WG	APXG	WG	APXG	WG	APXG
0.2	164.14^{a}	90.59^{a}	$1.3.10^{-3\ a}$	$8.1.\ 10^{-3\ a}$	0.30^{g}	$0.21^{e,f}$
0.3	$446.25^{a,b,c}$	$368.04^{a,b,c}$	$1.2.\ 10^{-3\ a}$	$6.7.\ 10^{-3\ a}$	$0.20^{e,f}$	$0.13^{c,d}$
0.4	$361.40^{a,b,c}$	$657.50^{a,b,c}$	$5.2.\ 10^{-3\ d}$	$8.1.\ 10^{-3\ a}$	$0.18^{d,e}$	$0.10^{b,c}$
0.5	$1924.4^{e,f}$	$1513.45^{d,e}$	$1.4.\ 10^{-3\ c}$	$5.2.\ 10^{-3\ a}$	$0.16^{d,e}$	0.03^{a}