

DEVELOPMENT OF STRUCTURED W/O EMULSIONS JUST WITH THE USE OF CANDELILLA WAX

Anaid de la Peña-Gil¹, Miriam Charo-Alonso¹, and Jorge Toro-Vazquez¹

¹Universidad Autonoma de San Luis Potosi

July 10, 2023

Abstract

We investigated the development of water-in-oil (W/O) emulsions just using CW, evaluating the effect of the water to CW oleogel ratio (40:60, 50:50, 60:40) and, at each ratio, the effect of the CW concentrations (0.75% to 3%). The emulsions were developed by shearing (60 s at 25°C) using an ultra-turrax type homogenizer. The emulsions were immediately evaluated and after 20 days of storage (25°C) for microstructure, water droplet diameter, emulsion stability through DSC freeze/thaw cycles, rheological properties, and X-ray measurements. The results showed that, at all water to oleogel ratios studied the CW developed structured W/O emulsions where the surface-active components of the CW (i.e., triterpenic alcohols, aliphatic alcohols, and fatty acids) stabilized the oil-water interface, while the n-alkanes and long chain esters formed an oleogel in the oil phase. Although, independent of the storage time, all the CW emulsions showed a frequency independent rheological behavior, after applying a strain above the G'¹-G'' cross point, the 40:60 and 50:50 emulsions with 1.5% to 3% CW concentration showed the better rheological behavior and were the most stables, even after two freeze-thaw cycles. In particular, the 40:60 and 50:50 emulsion with 1.5% CW had a recovery profile similar to commercial mayonnaise. In contrast, independent of the CW concentration, the 60:40 emulsions showed the lowest recovery profiles and higher instability to freeze-thaw cycles. These results indicated that the CW is a multi-functional material able to develop structured W/O emulsions useful for the formulation of trans-free, stable low-fat edible spreads.

DEVELOPMENT OF STRUCTURED W/O EMULSIONS JUST WITH THE USE OF CANDELILLA WAX

Anaid de la Peña-Gil, Miriam Charo-Alonso, Jorge F. Toro-Vazquez*

Facultad de Ciencias Químicas, Centro de Investigación y Estudios de Posgrado, Universidad Autónoma de San Luis Potosí, México

*Corresponding autor

Facultad de Ciencias Químicas-CIEP

Laboratorio de Fisicoquímica de Alimentos

Av. Dr. Manuel Nava 6

Zona Universitaria

San Luis Potosí, SLP 78290

México

e-mail: toro@uaslp.mx>

We investigated the development of water-in-oil (W/O) emulsions just using CW, evaluating the effect of the water to CW oleogel ratio (40:60, 50:50, 60:40) and, at each ratio, the effect of the CW concentrations (0.75% to 3%). The emulsions were developed by shearing (60 s at 25°C) using an ultra-turrax type homogenizer. The emulsions were immediately evaluated and after 20 days of storage (25°C) for microstructure, water droplet diameter, emulsion stability through DSC freeze/thaw cycles, rheological properties, and X-ray measurements. The results showed that, at all water to oleogel ratios studied the CW developed structured W/O emulsions where the surface-active components of the CW (i.e., triterpenic alcohols, aliphatic alcohols, and fatty acids) stabilized the oil-water interface, while the *n*-alkanes and long chain esters formed an oleogel in the oil phase. Although, independent of the storage time, all the CW emulsions showed a frequency independent rheological behavior, after applying a strain above the $G'-G''$ cross point, the 40:60 and 50:50 emulsions with 1.5% to 3% CW concentration showed the better rheological behavior and were the most stables, even after two freeze-thaw cycles. In particular, the 40:60 and 50:50 emulsion with 1.5% CW had a recovery profile similar to commercial mayonnaise. In contrast, independent of the CW concentration, the 60:40 emulsions showed the lowest recovery profiles and higher instability to freeze-thaw cycles. These results indicated that the CW is a multi-functional material able to develop structured W/O emulsions useful for the formulation of *trans*-free, stable low-fat edible spreads.

1. Introduction

The development of oleogels has emerged as a new and exciting field of lipid research. For food systems, the incorporation of edible oleogels allow the reduction of saturated fatty acids and the elimination of *trans*-fatty acids from the product (Dassanayake et al., 2011; Marangoni and Garti, 2018; Rogers et al., 2014) and in cosmetics the oleogels can be used as vehicles to delivery hydrophobic bioactive compounds (Ferrari and Mondet, 2003; Morales et al., 2009; Perez Nowak, 2011). Within this context, several vegetal waxes have gained considerable attention in the development of oleogels mainly because of their high gelling capacity and gel physical's properties, some even showing reversible thermomechanical properties. Additionally, the vegetable waxes are easy to obtain at affordable costs and most of them are already approved by the regulatory agencies (Blake et al., 2018).

Although most plant-based waxes are heterogenous mixtures of different components, their gelling capacity is usually associated with the major component that in the case of candelilla wax (CW) is the *n*-alkanes, i.e., 49%–50% *n*-alkanes with 29–33 carbons with hentriacontane as the *n*-alkane in major concentration (Grant, 2005; Nippo, 1985; Toro-Vazquez et al., 2007). Therefore, based on the *n*-alkanes concentration and gelling capacity of organic solvents (Abdallah and Weiss, 2000), our initial publications ascribed the high gelling capacity of the CW to the development of a three-dimensional crystal structure by the molecular self-assembly of the *n*-alkanes (Chopin-Doroteo et al., 2011; Morales-Rueda et al., 2009; Toro-Vazquez et al., 2007). However, experiments done in our laboratory showed that through a simple treatment extraction to reduce the concentration of long chain esters from the CW, resulted in a significant modification of the crystal habit of the oleogel, and also in a reduction of the gelling capacity and, subsequently, in an increase in the original minimal gelling concentration of CW (Romero Regalado, 2013). These results showed that the interaction among the native components of the CW determines its gelling properties, and the same concept applies to other vegetable waxes (Toro-Vazquez et al., 2023). On the other hand, a more in-deep CW analysis done using gas chromatography coupled with mass spectrophotometry, showed that besides *n*-alkanes the CW also has high concentrations of triterpenes, in particular triterpenic alcohols (i.e., [?] \times 23%) and esters of triterpenic alcohols (i.e., [?] \times 2%) (Ortega-Salazar, 2012). Triterpenes are a class of terpenes composed of six isoprene units characterized by a basic steroidal backbone with the $C_{30}H_{48}$ general molecular formula. Triterpenes are commonly present in several vegetables as triterpenoid glycosides or steroids, commonly referred to as saponins (Bottcher and Drusch, 2017; Wojciechowski, 2013). Terpenes and triterpenoid glycosides are compounds with well-known interfacial properties capable of developing foams (i.e., air-water interface activity) and oil-in-water emulsions (i.e., oil-water interface activity) (Liu et al., 2011; Pasureva et al., 2016; Sharma et al., 2023). On the other hand, some pentacyclic triterpenes present in several vegetable waxes (i.e., ursolic acid) have physical properties associated also with the development of organogels (Lu et al., 2019) and recently, it was reported that also are able to develop water-in-oil emulsions

stabilized through the Pickering effect (Liu et al., 2022).

Within the previous context and considering that the CW is constituted by components with molecular self-assembly and surface-active properties, this study evaluated the development of structured (i.e., gelled) water-in-oil (W/O) emulsions at room temperature (25degC) just with the use of the CW (i.e., absence of added surfactants). In a recent study, Penagos et al. (2023) developed W/O emulsions at 5degC using mixtures of beeswax and carnauba wax formulated with 20%, 30% and 40% (wt/wt) of water in sunflower oil. Based on contact angle measurement the authors discarded the CW, the berry wax, and the sunflower wax as tentative stabilizers of W/O emulsion. However, in this study no formal W/O emulsions were done to assess the emulsifying capacity of these vegetal waxes (Penagos et al., 2023). On the other hand, Garcia-Gonzalez et al. (2021) showed, through dynamic interfacial tension measurements, that in the temperature interval of 45degC to 60degC the CW significantly decreased the interfacial tension of safflower oil high in triolein from 26.4 (+- 0.9) mN/m to 5.9 (+- 0.5) mN/m upon the addition of 3% CW. These authors attributed this surface activity of CW to the adsorption of CW polar compounds (i.e., as fatty acids and triterpenic alcohols and triterpenic esters) on the water-vegetable oil interface (Garcia-Gonzalez et al., 2021). Although these authors did develop W/O emulsions (10% and 20% of aqueous phase) just with the use of 3% CW, the study made limited discussion regarding the CW emulsion's microstructure and stability (Garcia-Gonzalez et al., 2021).

In the present work, we hypothesized that after developing a CW oleogel, particular compounds of the wax (i.e., triterpenic alcohols, esters of triterpenic alcohols, long chain acids and alcohols) remaining in the oil phase, could act as surface-active agents with the capability of emulsifying a water phase forming a W/O emulsion. We considered that the CW oleogel present in the oil phase could provide a stabilizing mechanism for the emulsified phase, tentatively resulting in a structured W/O emulsion. Within this framework, the conditions investigated to develop the W/O emulsions were water to CW oleogel proportions of 40:60, 50:50, and 60:40 (wt:wt). The concentrations of the CW in the oleogels were selected so that, after the addition of water at the corresponding proportion, at each of the water to oleogel ratios studied the CW concentrations in the emulsions were 0.75%, 1.5%, 2.25%, and 3% (wt/wt). The W/O emulsions developed were evaluated for microstructure, water droplet size by NMR, emulsion stability by DSC, and rheological properties after 0 and 20 days of storage at 25degC.

2. Materials and Methodology

2.1. Materials.

The vegetable oil (VO) used was a refined, bleached, and deodorized high oleic safflower oil obtained from a local distributor (Coral Internacional, San Luis Potosi, Mexico). Previous characterization indicated that the major triacylglycerides (TAGS) in the VO used were OOO (65.65% +- 0.15%), LOO (16.26% +- 0.04), and POO (8.58% +- 0.04), and as minor TAGS components: StOO (2.64% +- 0.01%), LLO (2.25% +- 0.02%), POL (1.70% +- 0.11), StLL (0.87% +- 0.05%), and LLL (0.46% +- 0.01%) (O = oleic acid; L = linoleic acid; St = stearic acid; P = palmitic acid) (Alvarez-Mitre et al., 2012). The micronized refined candelilla wax (CW), supplied by Multiceras (Monterrey, Mexico) was previously characterized by gas chromatography coupled with mass spectrophotometry after derivatization of the CW with N,O-Bis(trimethylsilyl)trifluoroacetamide following the procedure described by Ortega-Salazar (2012). The results indicated that the main component of the CW were *n*-alkanes (45.75% +- 1.01), *n*-aliphatic alcohols (5.27 +- 1.40), *n*-fatty acids (15.17% +- 2.03), long chain esters (7.45% +- 0.61), triterpenic alcohols (23.41% +- 1.55), and esters of triterpenic alcohols (7.45% +- 0.17).

2.2. Development of the W/O emulsions

We developed CW solutions through the factorial combination of different weights of the VO (i.e., 40 g, 50 g, 60 g) and the CW (0.75 g, 1.5 g, 2.25 g, 3.0 g). The CW was fully dissolved in the oil by heating and intermittent stirring for 40 min in an oven set at 100degC. To develop the oleogels the CW solutions were allowed to cool until achieving 25degC (i.e., the cooling stage). For the oleogels formulated with 40 g of oil the %CW varied between 1.84% and 6.98%, for the oleogels formulated with 50 g of oil the %CW

varied between 1.48% and 5.66%, and for those formulated with 60 g of oil the %CW varied between 1.23% and 4.76%. Afterwards, the corresponding amount of deionized water (at 25degC) was added to make a total amount of 100 g. In this way, we obtained systems with water to oleogel weight ratios of 40:60, 50:50, and 60:40. With these systems we developed the emulsions by stirring for 60 seconds with an ultra-turrax type homogenizer (RIVAL Immersion blender Model IB900. The Holmes Group, Milford, MA) set at the higher speed (i.e., the emulsification stage). The resulting emulsions at each of the water to oleogel ratios studied, had CW concentrations of 0.75%, 1.5%, 2.25%, and 3%. The emulsions developed were analyzed immediately and after 20 days of storage at 25degC.

2.3. Polarized light microscopy

The microstructure of the emulsions was evaluated using a microscope (Olympus BX51, Olympus Optical Co., Ltd., Tokyo, Japan) equipped with a video camera (KPD50, Digital Hitachi, Tokyo, Japan), a heating/cooling stage (LTS 350, Linkam Scientific Instruments, Ltd.) connected to a temperature control station (TP94 and Linksys32 V 1.3.1; Linkam Scientific Instruments, Ltd., Surrey, England), and a tank of liquid nitrogen. Maintaining the temperature of the stage at 25degC we obtained images of the sample using polarized light.

2.4. Droplet size analysis

The water droplet size of the emulsions (25degC) was determined by NMR using the droplet size probe of a Minispec Bruker model mq20 (Bruker Analytik; Rheinstetten, Germany). The droplet size was measured in two independent samples (n = 2) reporting the diameter of the water droplets (μm) at the 97.5 percentile value (i.e., 97.5% of the droplets had a diameter equal or below this value, WDD_{97.5%}).

2.5. Emulsion stability through differential scanning calorimetry

The stability of the emulsions developed was evaluated through freeze-thaw cycles between 25°C and -70°C using a DSC (Discovery; TA Instruments, New Castle, USA). Initially, the emulsion samples (4 mg to 8 mg) sealed in hermetic aluminum pans were kept for 5 min at 25°C and then cooled down to -70°C (5°C/min). After 5 min at -70°C the sample was heated (5°C/min) until reached 25°C. After 5 at 25°C we repeated the freeze-thaw cycle under the same time-temperature conditions. The evaluation of the thermograms to determine the stability of the emulsions was done following the approach described by Clause et al. (2005) and Ghosh and Rousseau (2009).

2.6. Rheological properties of the emulsions

The mechanical properties of the emulsions were evaluated determining frequency (f) sweeps and time-dependent recovery measurements using a Discovery HR-3 Hybrid Rheometer (TA Instruments, New Castle, DE, USA) equipped with a stainless-steel parallel plates' geometry (40 mm diameter) with the surface of the upper plate sand-blasted. The temperature of the sample (25°C) was controlled using a Peltier system located on the base of the equipment. Initially, a sample of the emulsion (25°C) was placed on the base of the geometry preset at 25°C to then setting the upper plate to a gap of 1000 μm. Applying a constant strain (γ) we determined the f sweeps of the emulsion measuring the storage (G') and loss (G'') modulus. The γ applied varied between 0.06% and 0.10% as a function of the emulsion, but always was within the linear viscoelastic region (LVR) of the material previously established from the γ sweeps using a f of 1 Hz. Additionally, to establish the plastic region of the emulsions we did an onset point analysis of the G' values obtained from the γ sweeps determined using af of 1 Hz. With the elastic and plastic regions established we determined the time-dependent recovery behavior of the emulsions, initially applying during 60 s an γ within the elastic region (i.e., within the LVR) of the corresponding sample while measuring G' (G'_0). Afterwards, we applied during 30 s an γ within the plastic region (i.e., an γ above the G' - G'' cross point, γ^*) followed by the measurement of G' while applying during 300 s an γ within the LVR. From a master curve of the time-dependent recovery profile of two emulsions (n = 2), we calculated the instantaneous ($R_{10\text{ s}}$) and extended ($R_{300\text{ s}}$) elasticity recovery as:

$$R_{10\text{ s}} = (G'_{10\text{ s}}/G'_0)100 \text{ Equation 1}$$

$$R_{300\text{ s}} = (G'_{300\text{ s}})100/G'_{0\text{ s}} \text{ Equation 2}$$

where $G'_{10\text{ s}}$ and $G'_{300\text{ s}}$ corresponds to the G' mean values determined after 10 s and 300 s, respectively, of concluding the application of γ^* .

2.7. X ray analysis

We determined wide angle X-ray (WAX) diffractograms of selected CW oleogels, W/O emulsions, deionized water, and the CW using a Bruker diffractometer (Model D8 ADVANCE, Bruker Analytik, Rheinstetten, Germany) equipped with a Cu X-rays source ($\lambda = 1.5406 \text{ \AA}$). The measurements were done at 25°C obtaining angular scans from 1° to 40° using a step size of 0.01° at a scan speed of $0.0185^\circ/\text{sec}$. The analysis was performed using DIFFRAC.SUITE V7.5 software and the data processing using the DIFFRAC.EVA V5.1 software (Bruker Analytik, Rheinstetten, Germany).

2.8. Statistical analysis

To evaluate the effect of the different levels of water to oleogel ratio (40:60, 50:50, 60:40), the %CW in the emulsions (0.75%, 1.5%, 2.25%, 3%), and the storage time (0 and 20 days) on $WDD_{97.5\%}$ and G' , we establish a factorial design with two replicates ($n=2$). The statistical significance of the treatment variables' effect was determined through ANOVA, followed by *Student* -t test of linear combinations of the corresponding means (Statistica V 12.0; StatSoft, Tulsa, OK).

3. Results and Discussion

3.1. Microstructure and water droplet size behavior of the emulsions

Overall, independent of the water to oleogel ratio and the CW concentration used we obtained systems with a mayonnaise-like visual texture, easy to handle that showed no phase separation even after six months of storage at room temperature. As examples the Figure 1SM (Supplementary Material) shows pictures, after 20 days of storage at 25°C , of the W/O emulsions with 0.75% and 3% CW concentration developed at the different water to oleogel ratios studied. Figure 1 shows photographs obtained through polarized light microscopy (PLM) of W/O emulsions with 0.75% and 3% CW concentration developed at the different water to oleogel ratios studied with 0 days of storage (25°C). For comparison purposes the Fig. 1SM shows PLM photographs of the same emulsions as in Fig. 1 but after 20 days of storage at 25°C . From the visual analysis of the photographs, it was evident that, independent of the storage time, as the water to oleogel proportion increased the water droplets of the emulsions became larger (Figs. 1 and 1SM). Similar results were obtained with W/O emulsions with 1.5% and 2.25% of CW (data not shown). From the visual comparisons of the PLM photographs obtained with emulsions recently developed (i.e., 0 days of storage) with those after 20 days of storage, it was evident that, independent of the water to oleogel ratio, just the emulsions with 0.75% CW showed an increase in the droplet size after the 20 days of storage at 25°C (compare Fig. 1 and 1SM). This behavior indicated that at 0.75% the CW concentration was not enough to achieve an efficient emulsification of the water, and some coalescence occurred during the stirring and/or during storage. Nevertheless, none of these emulsions showed visual phase separation during their storage. In contrast, the PLM photographs of the W/O emulsions with 1.5%, 2.25%, and 3% CW at the different water to oleogel ratios studied, did not show a significant change in the water droplet size after the 20 days of storage (data not shown). The previous results were corroborated through the behavior observed by the $WDD_{97.5\%}$ in the W/O emulsions at 0 days and 20 days of storage (Fig. 2). Thus, as observed in the PLM photographs (Fig. 1), independent of the CW concentration used as the water to oleogel ratio increased the system developed emulsions with larger water droplets diameters (i.e., the $WDD_{97.5\%}$ increased). Additionally, the Fig. 2 showed that for a given CW concentration and water to oleogel ratio, the $WDD_{97.5\%}$ of the emulsions were statistically the same after 0 and 20 days of storage at 25°C . This $WDD_{97.5\%}$ behavior was observed even with the 0.75% CW emulsions at the different water to oleogel ratios (Fig. 2). Although the PLM of the 0.75% CW's emulsions showed an increase in the water droplet after storage (Figs. 1 and 1SM), it seemed that the NMR measurement of the water droplet diameter (i.e., the $WDD_{97.5\%}$) was not capable of detecting the tentative coalescence occurring during storage of the 0.75% CW emulsions. The $WDD_{97.5\%}$ results (Fig. 2) also showed that, independent of

the CW concentration, we developed emulsions of larger water droplet diameters as the water to oleogel ratio increased. We explained this behavior considering that as the water proportion increased the concentration of surface-active compounds from the CW became a limiting factor, simply because more water needed to be emulsified. The overall result was that we developed emulsions with larger water droplet diameters (i.e., higher WDD_{97.5%} value) as the water to oleogel ratio increased, particularly above the 50:50 water to oleogel ratio ($P < 0.05$; Fig. 2). A detailed statistical analysis of the WDD_{97.5%} behavior (Fig. 2) showed that, independent of the storage time of the emulsions, at the 40:60 water to oleogel proportion we required to increase the CW concentrations above 0.75% to achieve an additional reduction in the WDD_{97.5%} (i.e., decreasing the water droplet diameter) ($P < 0.05$). However, in the 50:50 emulsions the additional reduction in the water droplet diameter was achieved using CW concentrations above 1.5% ($P < 0.05$), and in the 60:40 emulsion just at a 3% CW ($P < 0.07$) (Fig. 2). It is important to note that increasing the CW concentration in the emulsions above these values did not result in an additional reduction in the WDD_{97.5%} value (Fig. 2). Then, the CW effect to decrease the emulsions' droplet diameter was lower as the water to oleogel ratio increased (Fig. 2). Consequently, at the highest proportion of water studied (i.e., 60:40), where the emulsions had the largest water droplet diameter, the effect of the CW to achieve lower WDD_{97.5%} was significant ($P < 0.07$) just using the highest CW concentration in the emulsions (i.e., 3%; Fig. 2). These results corroborate the conclusion that as the water proportion increased the CW concentration became the limiting factor for water emulsification. An additional factor that might limit the reduction of the water droplet diameter was that the shearing efficiency of the blender could decrease as the oleogels' hardness increased. Previous studies showed that the work of shear (i.e., the hardness) of 1% CW oleogels (25°C) increased from 37.18 g/mm (± 4.30 g/mm) up to 1455.54 g/mm (± 102.44 g/mm) in 3% CW oleogels (Toro-Vazquez et al., 2007). The CW concentration in the oleogels before adding the corresponding water proportion, had CW concentrations even above 3% (i.e., 4.5% and 6%). These CW concentrations would result in oleogels with even higher hardness than the previously reported in 3% CW oleogels (Toro-Vazquez et al., 2007), tentatively limiting the efficiency of the blender to reduce the water droplet diameter in the W/O emulsions.

3.2. Stabilizing mechanism of the W/O emulsions developed by the CW

The PLM photographs of the W/O emulsions included in Figs. 1 and 1SM showed that the water droplets were surrounded by a birefringent material. This birefringent material, indicated in the Fig.1 with black arrows, was uniformly distributed around the surface of all the water droplets. It is important to point out that none of the PLM photographs of the W/O emulsions showed the presence of wax crystals on the water-oil interface, indicating that the water droplets were not stabilized through the Pickering effect. These results contrast with the microstructure of the W/O emulsions formulated with 5% carnauba wax (40% water) or with 5% beeswax (20% water) developed through a pilot scale two-step process, consisting of a pre-emulsification step (90°C) followed by dynamic crystallization step (5°C) (Penagos et al., 2023). These authors showed through PLM and confocal laser scanning microscopy, that the water droplets of the carnauba wax and beeswax W/O emulsions were stabilized by wax crystals surrounding the droplets (i.e., Pickering effect) and by a crystal network developed in the oil phase by the corresponding wax (Penagos et al., 2023). On the other hand, as previously indicated pentacyclic triterpenes like the ursolic acid, can develop W/O emulsions tentatively stabilized also through the Pickering effect (Liu et al., 2022). Within this context it is important to note that the different stabilizing mechanisms observed in the emulsions developed in the present study with that reported by Penagos et al. (2023), might be associated with the different conditions used to develop the W/O emulsions and with the differences in wax composition. Carnauba wax consists mostly of long chain (C26 to C30) aliphatic esters ([?]40%) and diesters of 4-hydroxycinnamic acid ([?]21.0%), and a significant amount of long chain ω -hydroxycarboxylic acids ([?] 13.0%) and fatty alcohols ([?]12%) (Wolfmeier et al., 2016). Beeswax consists of [?]71% esters (mainly including [?]35% monoester, [?]14% diesters, [?]3% triesters, and [?]12% of hydroxymono- and hydroxypoly-esters), [?]14% *n*-alkanes, and [?]13% free fatty acids and alcohols (Tulloch, 1980). In the study of Penagos et al. (2023) the emulsification step was done at temperature conditions where the vegetable wax components were soluble in the oil phase. Under these conditions the surface-active molecules (i.e., fatty acids and fatty alcohols) of carnauba wax ([?] 25%) and beeswax ([?] 13%) would be adsorbed at the oil-water interface through their polar groups with

their aliphatic chains pointing toward the oil. We consider that because the molecular compatibility between the aliphatic chains of the adsorbed surface-active molecules and the long chain esters of the wax still in the oil solution, their nucleation and further crystallization on the oil-water droplet surface could occur during the cooling stage, followed by the additional crystallization in the continuous oil phase of the remaining long chain esters. The overall results would be that under the emulsifying and crystallization conditions used by Penagos et al. (2023) the carnauba wax and the beeswax developed O/W emulsions stabilized by Pickering and by a network of long chain ester crystals distributed through the continuous oil phase. In contrast, in the present study the emulsification was done at 25°C using mixtures of water and CW oleogels (i.e., 40:60, 50:50 and 60:40). The cooling thermograms included in Fig. 3 showed that, although most of the components of the CW were already crystallized at 25°C (temperature indicated with a dotted line in the thermograms of Fig. 3), still some CW components remained in the oil solution (i.e., required lower temperatures to crystallize in the oil phase). Within this context, the thermograms included in Fig.3 indicate the % of solid content achieved at 25°C (%SFC_{25°C}, determined by NMR) in the corresponding CW oil solution. With the values of %SFC_{25°C} we calculated the percentage of the CW that crystallized at 25°C in the oleogels (%CW_{25°C}). The corresponding statistical analysis showed that the %CW_{25°C} was statistically the same in all the CW oleogels, i.e., the %CW_{25°C} was the same independent of the CW concentration in the oleogel. The corresponding mean value of the %CW_{25°C} was 73.6% (± 5.0%). The %CW_{25°C} value mainly included the crystallization of the *n*-alkanes and long chain esters, components mainly involved in the development of the tridimensional crystal network of CW oleogels (Chopin-Doroteo et al., 2011; Morales-Rueda et al., 2009; Romero Regalado, 2013; Toro-Vazquez et al., 2007). However, the %CW_{25°C} value indicated that [?]26% of the CW components remained in the oil phase at 25degC. We considered that these CW components included the surface-active compounds involved in developing the W/O emulsion and, subsequently, forming the birefringent material present around the surface of all the water droplets (Fig. 1). Given the composition of the CW above reported (see section of “Materials”), these compounds tentatively involved the triterpenic alcohols, esters of triterpenic alcohols, aliphatic alcohols, and fatty acids. The PLM photographs of the CW emulsion also showed the presence of highly birefringent crystals in the oil phase (shown in Figs. 1 and 1SM with dashed arrows), particularly evident in the W/O emulsions with a final CW concentration of 3%. These birefringent microstructures, also present in CW oleogels, are characteristics of the crystals developed mainly by the co-crystallization of *n*-alkanes with long-chain esters. As a reference the Fig. 2SM includes PLM photographs of 1.5% and 3% CW oleogels developed following the same time-temperature and shearing conditions used in the development of the oleogels used for the development of the emulsions. The PLM photographs show the characteristics crystals found in CW oleogels (Fig. 2SM). From here we concluded that under the conditions used the systems developed by the CW were structured W/O emulsions where, tentatively, the triterpenic alcohols, esters of triterpenic alcohols, aliphatic alcohols, and fatty acids acted as surface-active agents at the oil-water interface, while the *n*-alkanes and long chain esters gelled the continuous oil phase.

3.3. Stability of the W/O emulsions developed by the CW

The stability of the W/O emulsions was evaluated through freeze-thaw cycles between 25degC and -70degC using a DSC. This methodology is particularly useful when considering that many types of edible W/O emulsions, such as whipped toppings and table spreads, are frozen to improve long-term storage and then thaw for further processing or consumption. Considering the concepts described by Clause et al. 2005 and Ghosh and Rousseau (2009), after freeze-thaw cycles stable emulsions ought to crystallize developing just one exotherm. For simplicity purposes we discuss just the behavior of the cooling thermograms obtained from the second freeze-thaw cycle (see section 2.5. Emulsion stability through differential scanning calorimetry) of the W/O emulsions after 20 days of storage at 25degC. Within this context, Figure 4 shows the cooling thermograms for the W/O emulsions developed at the different water to oleogel ratios used. The thermograms show the corresponding CW concentration in the emulsion. For comparative purposes Fig. 4 includes the cooling thermograms of the water and of the vegetable oil, obtained under the same time-temperature conditions as for the W/O emulsions. The corresponding exotherms had peak crystallization temperatures of -19.6degC (+- 0.3degC) and -45.1degC (+- 1.2degC) for the water and the vegetable oil, respectively (Fig.

4E). These thermograms were used as references to establish the tentative position of exotherms associated with the water or the oil released (i.e., “free”) from the microstructure of unstable W/O emulsions because of the freeze-thaw cycles. Within this context, the results shown in Fig. 4 indicated that the W/O emulsions having CW concentrations between 1.5% and 3% at water to oleogel ratios of 40:60 and 50:50, were the only ones that showed just one well-defined crystallization exotherm. This crystallization exotherm had, in all cases, a peak temperature at $[-40\text{degC}]$ (Fig. 4). The rest of the emulsions (i.e., 0.75% CW emulsions at all water to oleogel ratios, and the 1.5%, 2.25% and 3% CW emulsions at the 60:40 water to oleogel ratio) also showed the major exotherm with peak crystallization temperature $[-40\text{degC}]$ (Fig. 4). However, independent of the %CW, the emulsions developed with the 60:40 water to oleogel ratio also showed the presence of a large shoulder at temperatures above the major exotherm (indicated with a black arrow in the Fig. 4). In some emulsions, i.e., the emulsions with 0.75% CW at all water to oleogel ratios, we also observed a small shoulder at temperatures below the major exotherm (indicated with a dotted arrow in Fig. 4A). Considering the crystallization behavior of the water and the vegetable oil (Fig. 4E) and the concepts discussed for the characterization of W/O emulsions by DSC (Clausse et al., 2005; Ghosh and Rousseau, 2009), we associated the shoulder observed at a temperature above the major exotherm with “free” water, while the shoulder observed below the major exotherm with “free” oil. These “free” water and oil, released from the emulsion microstructure during the freeze-thaw cycles, were now dispersed throughout the still stable water droplets of the emulsion. From here and considering the results discussed for the PLM photographs (Figs. 1 and 1SM) and for WDD_{97.5%} (Fig. 2), we concluded that the W/O emulsions formulated with water to oleogel ratios of 40:60 and 50:50 and with CW concentrations between 1.5% and 3%, were the most stables even after two freeze-thaw cycles applied to the emulsions after storage for 20 days at 25degC.

3.4. X-ray analysis and rheology of the structured W/O emulsions

To have additional evidence of the microstructure of the systems developed, we obtained WAX diffractograms for 1.5% and 3% CW oleogels and the corresponding 1.5% and 3% CW W/O emulsions formulated with 40:60 and 60:40 water to oleogel ratios. The corresponding diffractograms are shown in Figure 5. As a reference to support the analysis of the diffractograms of the CW oleogels and the W/O emulsions, Fig. 5 includes the WAX diffractograms for deionized water (Figs. 5A and 5B) and CW (Fig. 5C). It is important to note that, except for the CW and the water, the diffractograms of the emulsions and the oleogels showed an amorphous signal with at peak at scattering that peaks centered at $d = 4.55 \text{ \AA}$ ($[2\theta] 19.5^\circ$). This amorphous signal was associated with the

liquid phase of triacylglycerols from the vegetable oil (Larsson, 1972). On the other hand, the diffractogram for the CW diffraction peaks at $2\theta = 21.5^\circ$ and $2\theta = 23.8^\circ$ corresponded to d values of 4.1 Å and 3.7 Å, respectively (Fig. 5C). These diffraction peaks are characteristic of the orthorhombic perpendicular subcell packing of the n -alkanes of the CW (Chopin-Doroteo et al., 2011; Dassanayake et al., 2009) and were also present in the 1.5% and 3% CW oleogels (Fig. 5D) and in the 1.5% and 3% CW emulsions formulated with 40:60 and 60:40 water to oleogel ratios (Fig. 5A and 5B). These results indicated that an oleogel microstructure, developed mainly by the n -alkanes and long chain esters of the CW, was present in the W/O emulsions. Additionally, the characteristic amorphous broad signal of the water with a peak at $2\theta [29^\circ]$ corresponding to a $d [3.15 \text{ \AA}]$ (Maciel et al., 2016) observed in the water diffractogram, was observed as a shoulder in the WAX diffractograms for the W/O emulsions formulated with 1.5% and 3% CW and water to oleogel ratios of 40:60 and 60:40 at $2\theta [29^\circ]$ ($d [3.16]$; Figs. 4A and 4B). This shoulder was larger and, subsequently, more evident in the emulsions formulated with the higher water proportion (i.e., 60:40 water to oleogel ratio). We considered that these results indicated the presence of a water phase confined throughout the microstructure of the oleogel. Based on these results and the ones obtained through PLM (Figs. 1 and 1SM) we consider that this water phase was emulsified, tentatively by the triterpenic alcohols, esters of triterpenic alcohols, aliphatic alcohols, and fatty acids. Therefore, the system studied was a W/O emulsion structured (i.e., stabilized) by an oleogel developed in the continuous oil phase by the n -alkanes and long chain esters of the CW.

The f sweeps of the W/O emulsions formulated with 1.5% and 3% CW concentrations at the different water to oil ratios studied after 20 days of storage are shown in Figure 3SM. Similar results were obtained with the W/O emulsions with 0.75% and 2.25% of CW (results not shown). All the emulsions studied showed a f independent rheological behavior, i.e., a gel-like rheological behavior. From the f sweeps of the emulsions, we obtained the corresponding G' value at an f of 1 Hz. From here we evaluated the elasticity of the W/O emulsions at 0 and 20 days of storage at 25degC as a function of the different water to oleogel ratios and CW concentrations used (Fig. 6). The results showed that, independent of the CW concentration, the G' of the emulsions increased as the water to oleogel ratio increased ($P < 0.05$), a behavior directly associated with the increase in the volume fraction of the emulsified water. Other studies also had shown that the increase in the volume fraction of the dispersed phase resulted in an increase of the emulsions' elasticity (Farah et al., 2005; Pal, 2006; Poling-Skutvik et al., 2020). Additionally, we observed that for the same water to oleogel ratio, the G' of the emulsions increased exponentially as a function of the CW concentration in the emulsions ($P < 0.05$). The G' increment was partly associated with a larger reduction in the water droplet diameter achieved as the CW concentration increased, an effect previously discussed regarding the $WDD_{97.5\%}$ behavior as a function of the CW concentration (Fig. 2). It is well-known that emulsions with smaller droplet size have higher elasticity (i.e., higher G') than emulsions with larger droplet size (Pal, 2006, 1996). Another factor associated with the G' behavior observed in the emulsions formulated at same water to oleogel ratio was that, as the CW concentration increased the hardness of the oleogel phase ought to increase. This behavior of the CW oleogels was previously reported by our group (Toro-Vazquez et al., 2007). Because the systems developed were W/O emulsions structured by the oleogel developed in the continuous oil phase, as the CW concentration increased, we obtained emulsions with a harder oleogel phase and, subsequently, emulsions of higher elasticity (i.e., higher G' , Fig 6). It is important to note that at all CW concentrations studied, after the 20 days of storage we observed a decrease in the elasticity of all emulsions. Nevertheless, independent of the CW concentration in the emulsion, the decrease in G' was significant just in the emulsions formulated with the 60:40 water to oleogel ratio ($P < 0.01$; Fig. 6). In the 40:60 and the 50:50 emulsions the storage time effect on the emulsions' G' was not significant at any of the %CW used (Fig. 6). These results corroborated that at the highest proportion of water utilized (i.e., 60:40 water to oleogel ratio), the amount of surface-active compounds present in the CW was insufficient to achieve an efficient emulsification of the water phase. Therefore, independent of the %CW used in the emulsions, we obtained larger water droplet diameters in the 60:40 emulsions (Figs. 1, Fig. 1SM, and Fig. 2) that resulted in emulsions with higher instability when compared with the 40:60 and 50:50 emulsions (Fig. 4).

As indicated in the methodology section, the $R_{10\ s}$ and $R_{300\ s}$ of the emulsions were determined from the corresponding time-dependent recovery profiles of the emulsions. The Fig. 4SM shows the time-dependent recovery master curves for the 1.5% and 3% CW emulsions developed at the different water to oleogel ratios. As a reference the Fig. 4SM-B indicates the points where $G'_{0\ s}$, $G'_{10\ s}$, and the $G'_{300\ s}$ were determined to calculate, using the Eqs. 1 and 2, the corresponding $R_{10\ s}$ and $R_{300\ s}$ of the W/O emulsions stored 0 and 20 days at 25degC. The corresponding $R_{10\ s}$ and $R_{300\ s}$ values were plotted as a function of the %CW and the water to oleogel proportion in the emulsions (Fig. 7). The results showed that, independent of the storage time, the emulsions with the highest $R_{10\ s}$ and $R_{300\ s}$ were those formulated between 0.75% and 2.25% CW in the emulsions using water to oleogel proportions of 40:60 and 50:50. In contrast, the emulsions developed with CW concentrations between 0.75% and 3% at the 60:40 water to oleogel proportion always had the lowest $R_{10\ s}$ and $R_{300\ s}$ values. These results indicated that the 60:40 emulsions, the ones with the larger water droplet diameters (Figs. 1 and 2), showed lower recovery capacity after deformation than the emulsions with a smaller water droplet diameter (i.e., the 40:60 and the 50:50 emulsions). From here and considering the previous results we concluded that the W/O emulsions formulated with water to oleogel ratios of 40:60 and 50:50 with CW concentrations between 1.5% and 3%, provided the better rheological behavior and were the most stables, even after two freeze-thaw cycles after storage for 20 days at 25degC.

4. Conclusions

A recent publication (Penagos et al., 2023) showed that carnauba wax and beeswax can develop structured W/O emulsions stabilized through the Pickering effect and by a network of wax crystal dispersed through

the oil phase. Based on contact angle measurement these authors discarded the CW as a tentative stabilizer of W/O emulsions, although under the conditions used (i.e., pre-emulsification step at 90degC followed by a dynamic crystallization step up to achieving 5degC) no W/O emulsions were done with the CW (Penagos et al., 2023). The results obtained in the present study showed that, under the time-temperature conditions used initially to develop a CW oleogel (i.e., cooling stage from 90degC to 25degC) followed by an emulsification process at 25degC, the CW can develop structured W/O emulsions with stability to freeze/thaw cycles even after 20 days of storage at 25degC. Under these processing conditions [?]26% of the CW components remained in the oil phase (i.e., triterpenic alcohols, esters of triterpenic alcohols, aliphatic alcohols, and fatty acids) and [?]73% of the CW components, mainly *n*-alkanes and long chain esters, developed an oleogel. We considered that the CW components remaining in the oil phase, could act as surface-active agents at the oil-water interface during the emulsification process stabilizing the water droplets. An additional stabilizing phenomenon of the CW emulsions was the hardness (i.e., elasticity) of the oleogel phase surrounding the water droplets. This oleogel, developed during the cooling stage before water addition, was structured through the crystallization of the constitutive *n*-alkanes and long chain esters of the CW. Consequently, a great extent of the rheological properties of the oleogel, essentially determined by the CW concentration, determined the elasticity (i.e., G') of the W/O emulsions (Fig. 6). Thus, at a constant water to oleogel ratio the G' of the emulsions increased exponentially as the CW concentration increased. An additional factor that determined the CW emulsions' rheology was the reduction in the water droplet diameter (i.e., decrease in the $WDD_{97.5\%}$ of the emulsions) associated with the emulsifying effect of the surface-active component of the CW. However, the emulsifying effect of the CW to decrease the $WDD_{97.5\%}$ of the emulsions (Fig. 2) that subsequently resulted in an increment in the emulsion's G' , depended on the water proportion in the emulsions. This was because, as the water fraction increased the amount of surface-active compounds of the CW became the limiting factor to achieve an efficient emulsification of the water phase. Consequently, when compared with the $WDD_{97.5\%}$ observed in the 40:60 and 50:50 emulsions, we obtained larger water droplet diameters (i.e., higher $WDD_{97.5\%}$ in the emulsions) in the 60:40 emulsions (Fig. 2). Therefore, independent of the CW concentration the emulsions developed at the higher water to oleogel proportion (i.e., 60:40), were also the ones showing the lower stability, as assessed by the freeze-thaw cycles applied by DSC (Fig. 4). The emulsions developed with the lower CW concentration, which represented the condition of most limiting concentration of surface-active compounds of the CW, also showed low emulsion stability that became more evident as the water to oleogel proportion increased (Fig. 4A). Our results indicated that the W/O emulsions formulated with water to oleogel ratios of 40:60 and 50:50 and with CW concentrations between 1.5% and 3%, were the most stable even after two freeze-thaw cycles applied emulsions stored for 20 days at 25degC. Finally, commercial standard and light mayonnaises observed similar rheological behavior than several W/O emulsions developed with the CW. Within this context, Figure 8 shows the time-dependent recovery profiles of commercial standard mayonnaise (M-1 with 46.4% \pm 0.2% water and 40.3% \pm 0.2% vegetal oil; M-2 with 12.0% \pm 0.1% water and 85.3% \pm 3.2% vegetal oil) (Fig. 8A) and commercial light mayonnaise (LM-1 with 61.3% \pm 1.3% water and 22.4% \pm 0.2% vegetal oil; LM-2 with 49.9% \pm 0.2% water and 17.4% \pm 4.7% vegetal oil) (Fig. 8B). The rheological profiles of these commercial mayonnaise are shown in comparison with the recovery profiles of structured W/O emulsions formulated with 40:60 and 50:50 water to oleogel ratios and 1.5% CW after 20 days of storage at 25degC. Evidently, the recovery profiles of the 1.5% CW emulsions formulated with water to oleogel ratios of 40:60 and 50:50 even after 20 days of storage, showed a rheological behavior closer to the ones observed by the standard mayonnaise (Fig. 8A). This in spite that, according to the mayonnaises' manufacturers, the water emulsification was done using highly efficient industrial homogenizers and stabilized with a combination of emulsifiers (i.e., egg yolk, whey protein, ovalbumin/egg white powder) and gelling agents (i.e., xanthan gum, modified starch). Consequently, when observed under the PLM the water droplets' diameter of the commercial mayonnaise was substantially smaller (data not shown) than the ones obtained with the blender used to develop the CW emulsions. The results of this study showed that CW is a multifunctional ingredient suitable for the elaboration of stable edible structured W/O emulsions. It is important to point out that under similar processing conditions other vegetable waxes (i.e., rice bran wax, carnauba wax) developed W/O emulsions. However, the emulsions developed by these waxes showed poor texture that after a few hours (i.e., rice bran wax) or after 3 to 4

days (i.e., carnauba) showed phase separation. In contrast, the mayonnaise-like W/O emulsions developed with CW concentrations between 1.5% and 3% using water to oleogel ratios of 40:60 and 50:50 did not have the waxy flavor characteristic of the CW oleogels, and were stable not showing phase separation even after 6 months at 25degC. Currently we are evaluating the development of CW emulsions utilizing a tabletop homogenizer under different time/temperature and shearing rate conditions using vegetable and mineral oil as the continuous phase.

5. Bibliography

Abdallah DJ, Weiss RG. n-Alkanes Gel n-Alkanes (and Many Other Organic Liquids). *Langmuir* 2000;16:352–5. <https://doi.org/10.1021/la990795r>.

Alvarez-Mitre FM, Morales-Rueda JA, Dibildox-Alvarado E, Charo-Alonso MA, Toro-Vazquez JF. Shearing as a variable to engineer the rheology of candelilla wax organogels. *Food Research International* 2012;49:580–7. <https://doi.org/10.1016/j.foodres.2012.08.025>.

Blake AI, Toro Vazquez JF, Hwang H-S. Wax Oleogels. In: Marangoni AG, Garti NBT-EO (Second E, editors. *Edible Oleogels*. 2nd ed., AOCS Press; 2018, p. 133–71. <https://doi.org/doi.org/10.1016/B978-0-12-814270-7.00006-X>.

Bottcher S, Drusch S. Saponins — Self-assembly and behavior at aqueous interfaces. *Advances in Colloid and Interface Science* 2017;243:105–13. <https://doi.org/10.1016/j.cis.2017.02.008>.

Chopin-Doroteo M, Morales-Rueda JA, Dibildox-Alvarado E, Charo-Alonso MA, de la Pena-Gil A, Toro-Vazquez JF. The Effect of Shearing in the Thermo-mechanical Properties of Candelilla Wax and Candelilla Wax-Tripalmitin Organogels. *Food Biophysics* 2011;6:359–76. <https://doi.org/10.1007/s11483-011-9212-5>.

Clausse D, Gomez F, Pezron I, Komunjer L, Dalmazzone C. Morphology characterization of emulsions by differential scanning calorimetry. *Advances in Colloid and Interface Science* 2005;117:59–74. <https://doi.org/10.1016/j.cis.2005.06.003>.

Dassanayake LSK, Kodali DR, Ueno S. Formation of oleogels based on edible lipid materials. *Current Opinion in Colloid & Interface Science* 2011;16:432–9. <https://doi.org/10.1016/j.cocis.2011.05.005>.

Dassanayake LSK, Kodali DR, Ueno S, Sato K. Physical properties of rice bran wax in bulk and organogels. *JAOCS, Journal of the American Oil Chemists' Society* 2009;86:1163–73. <https://doi.org/10.1007/s11746-009-1464-6>.

Farah MA, Oliveira RC, Caldas JN, Rajagopal K. Viscosity of water-in-oil emulsions: Variation with temperature and water volume fraction. *Journal of Petroleum Science and Engineering* 2005;48:169–84. <https://doi.org/10.1016/j.petrol.2005.06.014>.

Ferrari V, Mondet J. Care and/or Make-Up Cosmetic Composition Structured with Silicone Polymers and Organogelling Agents, in *Rigid Form* 2003:8.

Garcia-Gonzalez DO, Yanez-Soto B, Dibildox-Alvarado E, Ornelas-Paz J de J, Perez-Martinez JD. The effect of interfacial interactions on the rheology of water in oil emulsions oleogelled by candelilla wax and saturated triacylglycerols. *LWT* 2021;146:111405. <https://doi.org/10.1016/j.lwt.2021.111405>.

Ghosh S, Rousseau D. Freeze-thaw stability of water-in-oil emulsions. *Journal of Colloid and Interface Science* 2009;339:91–102. <https://doi.org/10.1016/j.jcis.2009.07.047>.

Grant DL. Candelilla wax (WHO food additive series 30). *INCHEM* 2005. <http://www.inchem.org/documents/jecfa/jecmono/v30je12.htm>.

Larsson K. Molecular Arrangement in Glycerides. *Fette, Seifen, Anstrichmittel* 1972;74:136–42. <https://doi.org/https://doi.org/10.1002/lipi.19720740302>.

- Liu CH, Chang FY, Hung DK. Terpene microemulsions for transdermal curcumin delivery: Effects of terpenes and cosurfactants. *Colloids and Surfaces B: Biointerfaces* 2011;82:63–70. <https://doi.org/10.1016/j.colsurfb.2010.08.018>.
- Liu Y, Xia H, Guo S, Lu X, Zeng C. Development and characterization of a novel naturally occurring pentacyclic triterpene self-stabilized pickering emulsion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 2022;634:127908. <https://doi.org/10.1016/j.colsurfa.2021.127908>.
- Lu J, Hu J, Liang Y, Cui W. The supramolecular organogel formed by self-assembly of ursolic acid appended with aromatic rings. *Materials* 2019;12:614. <https://doi.org/10.3390/ma12040614>.
- Maciel NR, Oliveira ECV, Okuma CH, Topan JF, Amaral LQ, Rocha-Filho P. A New System of Multiple Emulsions with Lamellar Gel Phases from Vegetable Oil. *Journal of Dispersion Science and Technology* 2016;37:646–55. <https://doi.org/10.1080/01932691.2015.1054506>.
- Marangoni AG, Garti N. *Edible oleogels: structure and health implications*. 2nd ed. Urbana, IL, United States: Elsevier, AOCS Press and Academic Press; 2018. <https://doi.org/10.1016/C2015-0-02413-3>.
- Morales-Rueda JA, Dibildox-Alvarado E, Charo-Alonso MA, Weiss RG, Toro-Vazquez JF. Thermo-mechanical properties of candelilla wax and dotriacontane organogels in safflower oil. *European Journal of Lipid Science and Technology* 2009;111:207–15. <https://doi.org/10.1002/ejlt.200810174>.
- Morales ME, Gallardo V, Clares B, Garcia MB. Study and description of hydrogels and organogels as vehicles for cosmetic active ingredients. *Journal of Cosmetic Science* 2009;60:627–36. <https://doi.org/10.1111/j.1468-2494.2010.00580.4.x>.
- Nippo Y (Central PAC). *The Japanese Standards of Cosmetic Ingredients*. 2nd ed. Yakuji Nippo; 1985.
- Ortega-Salazar MG. *Identificación de Compuestos Presentes en Cera de Candelilla Comercializada*. Universidad Autónoma de San Luis Potosí, 2012.
- Pagureva N, Tcholakova S, Golemanov K, Denkov N, Pelan E, Stoyanov SD. Surface properties of adsorption layers formed from triterpenoid and steroid saponins. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 2016;491:18–28. <https://doi.org/10.1016/j.colsurfa.2015.12.001>.
- Pal R. Rheology of high internal phase ratio emulsions. *Food Hydrocolloids* 2006;20:997–1005. <https://doi.org/10.1016/j.foodhyd.2005.12.001>.
- Pal R. Effect of Droplet Size on the Rheology of Emulsions. *AIChE Journal* 1996;42:3181–90. <https://doi.org/10.1002/aic.690421119>.
- Penagos IA, Murillo-Moreno JS, Dewettinck K, Van Bockstaele F. Carnauba Wax and Beeswax as Structuring Agents for Water-in-Oleogel Emulsions without Added Emulsifiers. *Foods* 2023;12:1850. <https://doi.org/10.3390/foods12091850>.
- Perez Nowak V. Fluid cosmetic composition, useful for making up and/or caring the skin and/or lips, comprises polyester, non-volatile silicone oil, organogelling agent comprising N-acylglutamides e.g. N-lauroylglutamic acid dibutylamide, and wax. 1154327, 2011.
- Poling-Skutvik R, Di X, Osuji CO. Correlation of droplet elasticity and volume fraction effects on emulsion dynamics. *Soft Matter* 2020;16:2574–80. <https://doi.org/10.1039/c9sm02394a>.
- Rogers MA, Strober T, Bot A, Toro-Vazquez JF, Stortz T, Marangoni AG. Edible oleogels in molecular gastronomy. *International Journal of Gastronomy and Food Science* 2014;2:22–31. <https://doi.org/10.1016/j.ijgfs.2014.05.001>.
- Romero Regalado JI. *Bioceras: Fraccionamiento en Fase Humeda*. Universidad Autónoma de San Luis Potosí, 2013.

Sharma K, Kaur R, Kumar S, Saini RK, Sharma S, Pawde S V., et al. Saponins: A concise review on food related aspects, applications and health implications. *Food Chemistry Advances* 2023;2:100191. <https://doi.org/10.1016/j.focha.2023.100191>.

Toro-Vazquez JF, Aguilar-Zarate M, Charo-Alonso MA. Vegetable waxes as multicomponent gelator systems. In “Advances in Oleogel Development: Characterization and Nutritional Aspects.” 1 St. Switzerland: Springer Nature; 2023.

Toro-Vazquez JF, Morales-Rueda JA, Dibildox-Alvarado E, Charo-Alonso M, Alonzo-Macias M, Gonzalez-Chavez MM. Thermal and textural properties of organogels developed by candelilla wax in safflower oil. *JAOCs, Journal of the American Oil Chemists’ Society* 2007;84:989–1000. <https://doi.org/10.1007/s11746-007-1139-0>.

Tulloch AP. Beeswax—Composition and Analysis. *Bee World* 1980;61:47–62. <https://doi.org/10.1080/0005772X.1980.11097776>.

Wojciechowski K. Surface activity of saponin from Quillaja bark at the air/water and oil/water interfaces. *Colloids and Surfaces B: Biointerfaces* 2013;108:95–102. <https://doi.org/10.1016/j.colsurfb.2013.02.008>.

Wolfmeier U, Schmidt H, Heinrichs F-L, Michalczyk G, Payer W, Dietsche W, et al. *Waxes* 2016;5:1–204. <https://doi.org/10.1002/14356007.a28>.

Hosted file

Fig 1.docx available at <https://authorea.com/users/456623/articles/653849-development-of-structured-w-o-emulsions-just-with-the-use-of-candelilla-wax>









