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Article

Complexed Biopolymer of Whey Protein and Carboxymethyl Cellulose to Enhance the Chemical Stability of Lemon Oil-in-Water Emulsions

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ABSTRACT: Lemon oil (LO) has been used as a flavoring food additive and its encapsulation in emulsions has facilitated its incorporation in food and pharmaceutical products. D-Limonene, the main ingredient in LO, and citral, the compound responsible for the aromatic profile, are easily oxidized and a new emulsion interfacial structure is needed to protect these compounds. This paper studies the physical and chemical stabilities of LO-in-water emulsions produced by membrane emulsification as a function of the oil droplets' surface electric charge and interfacial thickness. For that purpose, the different stabilizers used were whey protein (pH: 3.8 and 6.8), and, for the first time in lemon oil encapsulation, an electrostatic whey protein−carboxymethyl cellulose (WP-CMC) complex (pH: 3.8). Although this complex did not improve emulsions' physical stability when it comes to droplet size, it has contributed to maintaining the emulsions' original color. As for chemical stability, the WP-CMC complex decreased the production of off-flavors resulting from the deterioration of D-limonene and citral under accelerated oxidation conditions.

KEYWORDS: *SPG membrane emulsification, whey protein, electrostatic complex, physical/chemical stability*

1. INTRODUCTION

Essential oils are known to deteriorate quickly producing off-flavors and by that reducing their shelf life.[1] Lemon oil, a natural flavoring and food additive, which consumers nowadays highly seek, is rich in unsaturated and oxygen functionalized terpenes making it susceptible to oxidation caused by oxygen, light, and heat. The main components of lemon oil are D-limonene, γ-terpinene, β and α-pinene, and the isomers of citral, neral, and geranial (Table S1 in Supporting Information). Neral and geranial dominate the overall flavor profile of fresh lemon oil over more than 130 volatiles found in lemon oil.[2]

D-Limonene is the main constituent of lemon oil making up 45−80% of it. This cyclic monoterpene is susceptible to degradation by acid-catalyzed hydration-dehydration and oxidation reactions. Byproducts of these reactions include carvone, carveol, 2,8-menthadiol, and limonene oxide (Figure 1).[3] Likewise, citral, a monoterpene aldehyde and key contributor to citrus flavor, decomposes rapidly during storage at acidic pH by a series of cyclization and oxidation reactions (Figure 1). As a result of citral degradation, the level of fresh lemon-like aroma decreases, and off-flavors are generated. To reduce the occurrence of these reactions and extend the self-life of lemon oil, a protection technique is needed.

Lemon oil, and citrus oils in general, have been used for flavoring purposes of soft drinks, dairy products, and confectionaries. Because citrus oils are not majorly water-soluble, they are added to many foods and beverages as oil-in-water (O/W) emulsions, which in turn become a way of protecting volatile and/or active ingredients. Many studies have examined some of the major factors influencing the production and stability of O/W emulsions encapsulating lemon oil, citral, or limonene.[4] Recently the impacts of high- and low-energy emulsification technologies on the properties of lemon oil emulsions have been reported. However, little to no information is present on process productivity. While high-energy technologies, such as high-pressure homogenization or sonication, apply high shear forces and cavitation to obtain stable lemon oil emulsions with a droplet size that can be below 100 nm,[5,6] low-energy emulsification technologies, particularly membrane emulsification, produce emulsions with a narrow droplet size distribution at low shear stress conditions.[7,8]

Moreover, the role of interfacial properties of lemon oil emulsions in controlling their physical and chemical stability as well as the release of encapsulated aroma compounds has been widely investigated. Special attention has been paid to biopolymers because of their capacity to both form layers/coatings able to stabilize the oil−water interface and reduce the access of H^+ and prooxidant metals to the interface and by that, the conditions promoting chemical degradation. Accordingly, whey proteins (WPs) were found to decrease oxidative reactions in citral emulsions at pH 3 and that was linked to their ability to form a cationic emulsion interface able to repel pro-oxidative metals and/or the presence of amino acids in whey proteins

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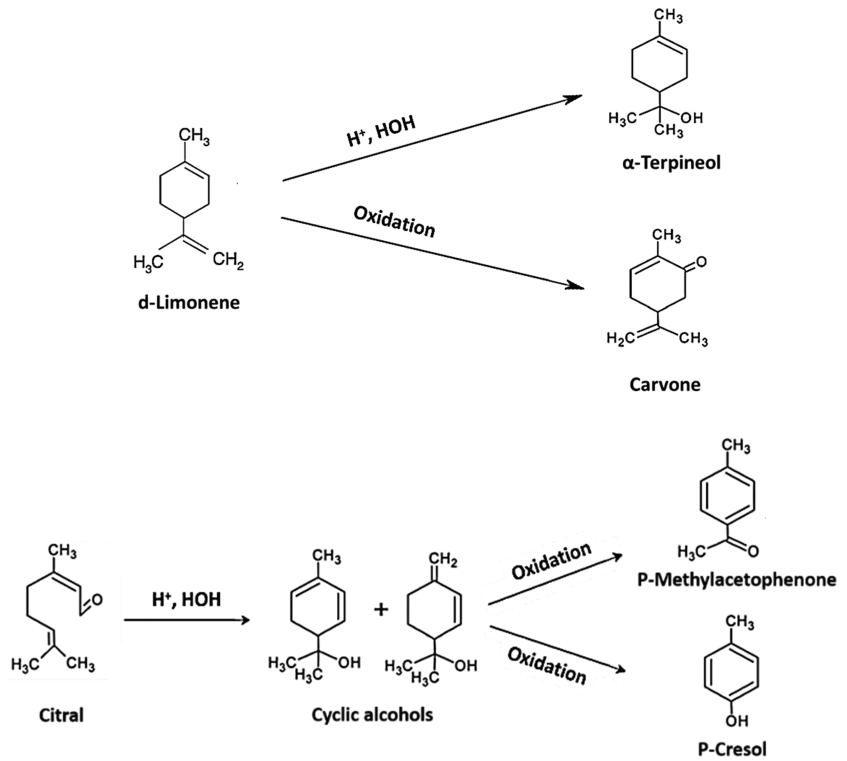

Figure 1. Degradation pathways of D-limonene and citral (Adapted with permission from refs 3 and 28. Copyright 2014 Royal Society of Chemistry and Copyright 2005 Springer-Verlag).

capable to scavenge free radical and chelate pro-oxidative metals.[1]

More recently, engineered interfaces with proteins combined with polysaccharides have been explored to control chemical degradation in citral, limonene, and lemon oil emulsions. Maillard conjugates of sodium caseinate and lactose[3] partially inhibited off-flavor formation from lemon oil emulsions compared to those stabilized with the single protein, while soy protein−polysaccharide Maillard conjugates improved the physical stability of citral emulsions during prolonged storage.[9] Furthermore, interfacial stabilization using the layer-by-layer technique, in which oppositely charged polymers, such as proteins and polysaccharides are adsorbed onto the droplet surface to create a bilayered interfacial membrane, has produced stable emulsions with better control of lemon oil degradation. In particular, dairy proteins, e.g. lactoferrin, α-lactalbumin, β-lactoglobulin, or sodium caseinate, were used to absorb at the droplet surface while anionic polysaccharides like pectin or gum Arabic, were added to the system to electrostatically interact with the already positively charged interface.[10−12] Likewise, lecithin, which works as an anionic emulsifier because of the functional head groups of various phospholipids, has been used to create negatively charged droplets subsequently coated by positively charged polysaccharides such as chitosan.[9] Although the electrical surface charge of the oil droplets has been suggested as an efficient strategy to reduce the access of prooxidant cations to the interface,[13] it may not be the only factor to consider seeing as both negatively and positively charged interfacial bilayers have been found to reduce the chemical degradation of citral and limonene emulsions. Apparently, the thicker interfacial bilayer formed by proteins and polysaccharides, as well as the enhanced viscosity of the continuous phase in these emulsions contribute to preventing the access to pro-oxidant cations to the interface.

An alternative approach to stabilize lemon emulsions with thick interfacial layers made of protein and polysaccharides is through using electrostatic complexes as stabilizers. Soluble protein−polysaccharide complexes, resulting from electrostatic interactions between oppositely charged macromolecules at certain conditions of pH and ionic strength, have been successfully used to stabilize oil-in-water interfaces in single O/W[14,15] and multiple W/O/W emulsions.[16,17] Berendsen, Güell, Henry, and Ferrando[18] reported that sunflower O/W emulsions stabilized with a whey-protein−carboxymethyl cellulose (WP-CMC) electrostatic complex maintained a more stable droplet size distribution than O/W emulsions stabilized with a monolayer of WP or those stabilized by a bilayer made of positively charged WP coated by negatively charged CMC. Nevertheless, sunflower oil oxidized much faster in emulsions stabilized with a WP-CMC electrostatic complex than in those with just WP, which was attributed to the negatively charged droplet surface obtained with the electrostatic complex.

This work aimed to determine how both the surface electric charge and the interfacial thickness impact the physical and chemical stability of lemon O/W emulsions when stabilized with WP at neutral and acidic conditions and, for the first time, a WP-CMC electrostatic complex. To do so, emulsions were obtained using the well established premix Shiratsu porous glass (SPG) membrane emulsification. To assess to what extent each interfacial structure affected the physical and oxidative stability of O/W emulsions, droplet size distribution and the evolution of some key components of the lemon oil volatile profile at accelerated oxidative conditions were monitored over 2 weeks.

2. MATERIALS AND METHODS

2.1. Materials. To produce O/W emulsions, distilled water was used and the lemon oil (cold- pressed) was purchased from Dallant, Spain. The stabilizers used were whey protein (Davisco Foods

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ACS Food Sci. Technol. 2022, 2, 41−48

Table 1. Membrane's Properties and Coarse Emulsion Characterization

					coarse emulsion	
membrane	membrane pore size (μm)	emulsifier	pressure (kPa)	oil fraction (%)	$d_{3,2}$ (μm)	span (−)
SPG	1	1% WP (pH: 6.8)	850	20	13.87 ± 0.55	0.99 ± 0.02
		1% WP (pH: 3.8)	850	20	13.76 ± 0.15	0.99 ± 0.02
		0.5% WP−0.25% CMC (pH: 3.8)	850	20	19.38 ± 0.49	0.98 ± 0.04

International, USA) and a protein−polysaccharide electrostatic complex: whey protein−carboxymethyl cellulose. CMC (Sigma-Aldrich, San Luis, Missouri, CAS 900-32-4) was purchased from Sigma-Aldrich Quimica SL, Spain. Hydrochloric acid (HCl) was purchased from Fisher Scientific (UK).

2.2. Whey Protein and Electrostatic Complex Preparation. The continuous phase containing whey protein was prepared 1 day before using it for emulsification and stored in the refrigerator (4 °C) overnight. The three stabilizers used in this study were 1 wt % WP (pH 6.8), 1 wt % WP (pH 3.8), and 0.5 wt % WP−0.25 wt % CMC electrostatic complex (pH 3.8). A 200 mL portion of 2 wt % WP solution was diluted on the day of the experiment to 1 wt %. Upon dilution, the pH of the 1% WP solution was 6.8, while for the WP that needed pH modification to 3.8, it was first diluted with a 20 mM acetic acid buffer then 1 M HCl was added until reaching the desired pH valued. As for the WP-CMC complex, 2 wt % WP, and CMC solutions, they were prepared the day before. On the day of the experiment, 200 mL of the complex was prepared by mixing the following solutions: 22.5 g of 2 wt % CMC, 22.5 g of distilled water, 90 g of 20 mM acetic acid buffer, and finally 45 g of 2 wt % WP. At this point, the pH of the complex was adjusted to 3.8 with 1 M HCl. Berendsen et al.[18] have characterized this complex and shown that this protein−polysaccharide 2:1 proportion is soluble at pH 3.8 and that the complex is negatively charged (more details in section 3.1).

2.3. Premix Membrane Emulsification. Lemon oil coarse emulsions (200 g) to be refined with SPG membranes had a 20% oil fraction. For emulsification, a high-speed mini kit, with a 1 μm hydrophilic SPG membrane (tubular membrane of 125 mm length × 10 mm external diameter × 0.8 mm wall thickness, SPG Technology Co., Japan) was utilized. Table 1 details the experimental conditions and combinations tested in this paper. All coarse emulsions (a.k.a premix) were first homogenized for 3 min with a high shear mixer (IKA T-18 basic Ultraturrax) at 15 500 r.p.m with a 30 s break after each minute (2 breaks in total) to avoid overheating. A 1 mL sample of the coarse emulsions was then taken for droplet size distribution analysis as described in section 2.5.

To refine the coarse emulsion, it was placed in a pressure tank and pushed with nitrogen gas through the membrane at 850 kPa. Emulsions were passed through the SPG membrane three consecutive times (three cycles) to further refine the emulsions. After each cycle, a sample was taken for droplet size distribution analysis.

2.4. Membrane Cleaning. The SPG membrane, after it was used for emulsification, was soaked and sonicated in acetone for 15 min then placed in a furnace at 500 °C for 8 h. To restore the hydrophilicity of the membrane, it was soaked and sonicated in 2 N HCl for 1 h. Then, the membrane was sonicated in distilled water, three times, for 30 min, changing the water every time.

2.5. Droplet Size Distribution Measurement. Emulsion characterization consisted of droplet size and droplet size distribution analysis using laser diffraction in a Malvern Mastersizer 2000 equipped with a Hydro200SM module. The emulsion sample was diluted to a 1:10 ratio and three measurements were performed in the Mastersizer. For each sample introduced (measurement), the Mastersizer performs three readings. This resulted in nine total readings for each sample. An average of all readings was calculated, and the area-volume mean diameter or Sauter mean diameter ($d_{3,2}$) and the droplet span were calculated by the software.

2.6. Zeta-Potential Measurement. Laser doppler microelectrophoresis (Zetasizer Nano ZS, Malvern Instruments, Ltd., Worcestershire, UK) was used to measure ζ-potential of all the emulsions produced in the present study, that is lemon oil droplets stabilized with 1 wt % WP (pH 6.8), 1 wt % WP (pH 3.8), and 0.5 wt % WP−0.25 wt % CMC electrostatic complex (pH 3.8). ζ-Potential is the result of net electric charge between two fluid planes. Measurements were done by diluting 0.5 mL emulsions sample with 20 mL of the acetic acid buffer. All measurements, for fresh emulsions and the ones for the stability study, are an average of nine measurements calculated by the Smoluchowski equation.[19]

2.7. Emulsion Stability under Accelerated Oxidation Conditions. Every emulsion prepared was divided into ten glass tubes of 12 mL. Five of these tubes were used for physical stability measurements, and the other five are for the chemical stability measurements. For an accelerated stability test, all ten of the tubes were placed in a closed wooden box that contains a UV light (Philips TL 8*W*/108 ultraviolet) source on the inside.[20] The box also had a thermometer inside to monitor the temperature and the temperature was on average 25 ± 2 °C. This technique is similar to that reported by Guan et al.[21]

On a sampling day, two tubes were removed from the box (one for physical and the other for chemical stability measurements). Sampling was done after 2, 3, 7, 10, and 14 days.

2.7.1. Physical Stability. The physical stability of the emulsions was followed by taking pictures of the emulsions over time noting any color changes (appearance of a yellow tint that usually accompanies oxidation) or form (a form of destabilization like creaming or phase separation). Also, the droplet size distribution was measured as described in section 2.5, and the ζ-potential was measured as described in section 2.6.

2.7.2. Chemical Stability. The emulsions' chemical stability was measured by evaluating the volatile profile evolution. The analytical technique used was gas chromatography (GC) (Varian, Middelburg, The Netherlands) so a pretreatment of the emulsion was necessary to separate the volatile fraction. Among the different sample preparation techniques, the most appropriate to the present study was the solid-phase microextraction applied to the headspace of the sample (HS-SPME).

Before the analysis of the samples and to obtain and ensure reproducible results, the different parameters that affect the micro-extraction process were optimized: temperature and time of extraction, sample volume, and fiber coating. The best results were obtained when the analyses were performed as follows:

(1) A 0.380 ± 0.005 g portion of the homogeneous emulsion was diluted in a 100 mL volumetric flask and homogenized using a vortex.

(2) A 0.250 mL amount of this diluted sample was placed in a 20 mL glass vial with a magnet bar inside.

(3) A 10 mL portion of an 8% sucrose solution was added to the vial, it was then sealed hermetically with a PTFE/silicone septum and finally shaken manually to homogenize the mixture.

(4) The vial was thermostated at 40 °C under constant medium stirring for 15 min to promote the volatile compounds to move to the headspace.

(5) A 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane mixture on a 2 cm StableFlex fiber was then inserted into the headspace of the vial by using a special syringe device. The fiber was kept in the headspace for another 15 min to extract the volatile compounds.

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ACS Food Sci. Technol. 2022, 2, 41−48

(6) The syringe needle that houses the fiber was inserted into the gas chromatograph injection port, and once there, the fiber was exposed at 270 °C for 3 min to guarantee the release of all the volatile compounds into the GC.

Regarding the GC separation, it was performed using a gas chromatograph (Hewlett-Packard 5890) equipped with a flame ionization detector (FID). The sample injection was carried out in splitless mode for 1 min and using an inlet of 0.75 μm I.D. to minimize peak broadening. Chromatographic separation was made using an HP-5 M.S. (Cross-linked 5% Ph Me Silicone) (30 m × 0.25 mm I.D., 0.25 μm film thickness) fused silica capillary column. The carrier gas was helium (1.5 mL/min) and the FID detector was supplied with air (447 mL/min), hydrogen (40 mL/min), and helium (22 mL/min). The oven temperature was set at 40 °C for 2 min, then increased to 200 °C at 20 °C/min, and finally at 50 °C/min to 275 °C (50 °C/min) temperature that was held for 7 min. The temperature of the detector was maintained at 200 °C.

The supplier of lemon oil provided a chromatographic profile obtained when analyzing it by GC-FID together with the identification of the peaks. Upon reception of the lemon oil, these identities were verified by using a gas chromatograph (Hewlett-Packard 6890) coupled to a mass selective detector Hewlett-Packard HP-5973. Separation was achieved under the same conditions above-described and using the same column used in the GC-FID analysis to identify the odorant compounds using the retention time parameter. The mass spectrometer operated in the electron impact ionization mode (70 eV). Interface, source, and quadrupole temperatures were 250, 230, and 150 °C, respectively. The mass range was from 35 to 300 amu, and the mass spectra library used was NIST14.L.

To monitor the evolution of the different lemon oil emulsions, the chromatographic response of each peak corresponding to α-terpineol, neral, carvone, and geranial was considered. Specifically, the change (either an increase or a decrease) of each peak (in percentage) was evaluated by comparing the peak area on the sampling day (A_t) versus that obtained on the day of the emulsification (A_0) according to eq 1.

$$\text{variation (\%)} = \frac{A_t}{A_0} \times 100 \quad (1)$$

3. RESULTS AND DISCUSSION

3.1. Properties of Lemon O/W Emulsions Produced with SPG Membranes. Coarse emulsions containing 20 wt %. (referred to the total emulsion) lemon oil and stabilized with 1 wt % WP (pH 6.8), 1 wt % WP (pH 3.8), and 0.5 wt % WP−0.25 wt % CMC electrostatic complex (pH 3.8) were refined by premix ME with the SPG membrane. The droplet size reduction and reduction patterns were similar for all of them: the highest droplet breakup occurred in the first emulsification cycle (69% size reduction) with little to no change in the consecutive cycles (Figure 2). As for the span, it has improved increasing the number of cycles only for emulsions stabilized with WP, reaching a span of 0.8 after the third cycle. The span of the refined emulsions produced with the WP-CMC complex, however, was higher (≈1.37) than that of the coarse emulsion (≈0.98) indicating a less efficient capacity of the WP-CMC complex either to reduce interfacial tension or to reach the oil-in-water interface during the emulsification process.

Berendsen et al.[18] have produced 20 wt % sunflower oil-in-water emulsions with 10 μm SPG membrane and stabilized with 1 wt % WP at pH of 3.8 and 0.5 wt % WP−0.25 wt % WP-CMC at pH of 3.8. These authors report a similar trend in droplet breakup to the one obtained in this study; however, the span values they report were much higher (1.7 for WP and 3.6 for WP-CMC). This might be due to the higher viscosity of sunflower oil or also that their SPG membrane had larger pore sizes. Bigger sizes of the membrane pores may lead to different

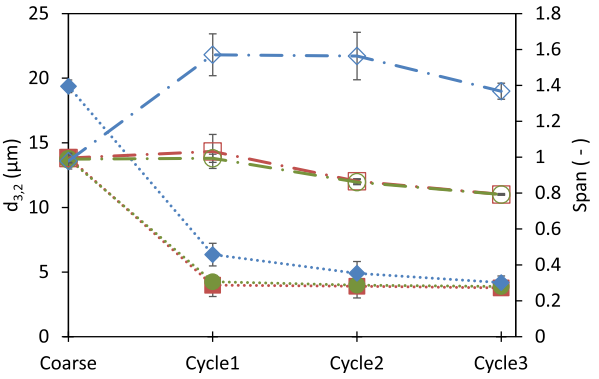

Figure 2. Effects of emulsification cycles and emulsifier type on oil droplet size ($d_{3,2}$) and span. (■, □) WP-pH 6.8, (●, ○) WP-pH 3.8, (♦, ◊) WP-CMC-pH 3.8. Filled markers refer to droplet size, and empty markers refer to droplet span

mechanisms of droplet breakup and further recoalescence, thus leading to less monodisperse emulsions.

Regarding zeta potential, all measured ζ-potential distributions gave a monomodal distribution. From Figure 3c, it is clear that ζ-potential depended on the interfacial composition. Lemon oil emulsions stabilized with 1 wt % WP at pH 6.8 exhibited a negatively charged surface (isoelectric point of WP is 5.2) of −48 mV, while at pH 3.8 the interface was positively charged with a ζ-potential of +24 mV. In contrast, lemon oil droplets in emulsions stabilized with 0.5 wt % WP−0.25 wt % CMC electrostatic complex at pH 3.8 showed a negative droplet surface charge of −41 mV. In a previous work, Berendsen et al.[16] found that water-in-oil-in-water (W/O/W) emulsions formulated with sunflower oil and 0.5 wt % WP−0.25 wt % CMC electrostatic complex at pH 3.8 as hydrophilic emulsifier, had a ζ-potential of the oil droplets of −24 mV. Also, measuring the ζ-potential of the WP-CMC complex in an aqueous solution (before using it for emulsification), Berendsen et al.[18] gave a value of −28 mV, which is quite close to that of the W/O/W emulsion. This difference between the ζ -potential of lemon and sunflower oil droplets stabilized by the same WP-CMC complex at pH 3.8 may be attributed to the variations in the composition and polarity of the oil phases. In fact, a recent study producing 20 wt % lemon oil emulsions, showed that lemon oil is capable of lowering an emulsion's pH thus altering the ζ-potential of the droplets' interfaces.[22] According to the authors, the aromatic lemon oil, and unlike sunflower oil, contains water-soluble compounds (mainly anhydrous and phenolic acids) that lower the emulsion pH and by that changing the ζ-potential.

3.2. Physical Stability of Lemon O/W Emulsions Produced with SPG Membranes. The stability of lemon oil emulsions was examined for 14 days of storage at accelerated oxidation conditions induced by UV light. For what regards physical stability, droplet size distribution, zeta-potential, and color change were monitored. The emulsions stabilized with WP-pH 3.8 seem to maintain the same droplet size over the whole stability period but the size dispersion started increasing linearly after 3 days (Figure 3a,b). Moreover, emulsions stabilized with WP in acidic conditions showed higher stability when compared to those stabilized with WP at pH 6.8. The latter reached a span value after 14 days that is five times the original value. This observation goes in the same direction as that of Djordjevic et al.[1] when comparing the effect of emulsifier pH.[1] These authors found that during homogenization, emulsions stabilized by WP at pH 7 showed a smaller droplet size

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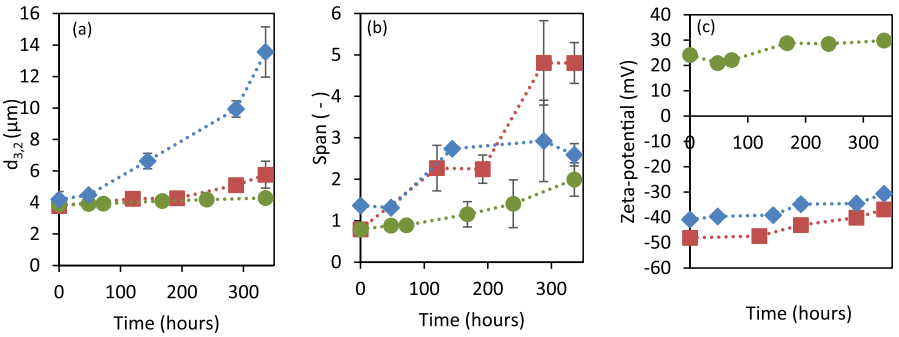

Figure 3. Physical stability of emulsions with (■) WP-pH 6.8, (●) WP-pH 3.8, and (♦) WP-CMC-pH 3.8 interfaces in terms of (a) droplet size, (b) span, and (c) z-potential.

distribution than those at pH 3. However, at pH 7 the mean particle diameter increased considerably after 1 day of storage, which was attributed to droplet flocculation caused by an increase in the surface hydrophobicity of the lipid droplets when the adsorbed proteins underwent surface denaturation. Another reasoning could be the formation of disulfide bonds between adsorbed proteins on different oil droplets.[23] McClements et al.[24] report that WP stabilizing O/W droplets may interact with WP molecules on neighboring droplets or even a single WP molecule adsorbing to two oil droplets. This interaction leads to the formation of disulfide bonds.

As for emulsions stabilized with the electrostatic complex, Figure 3a,b show that droplet size distribution and span started increasing after 48 h. With a span value ≈1.4, the emulsion already had a somewhat polydisperse droplet size distribution from the first day. This is certainly a factor in increasing the potential of coalescence. After 14 days the span of the emulsion was ≈2.6 which is in line with the value (≈2.5) reported by Berendsen et al.[18] for sunflower oil emulsions after 14 days under UV light. These results are in the range of those shown by Su et al.[11] for O/W emulsions containing D-limonene and stabilized with a bilayer made of β-lactoglobulin and gum arabic, in which after 2 weeks of storage at 25 °C the droplet size increased almost doubled compared to the initial value. Similarly, Xiang et al.[10] reported that the physical stability of citral emulsions coated by an interfacial bilayer formed by whey proteins and pectin was lower than that found in single-layered emulsions.

Regarding a visual assessment of stability, all the emulsions underwent creaming. This meant that, once a sample was collected for physical or chemical stability measurements, the emulsions were manually and gently homogenized by repetitively rotating the tubes. Another interesting factor to be noted is the yellow color evolution in the emulsions. The emulsion with the most popping color change is the one stabilized with WP-pH 6.8 (Figure 4a). The emulsion stabilized with WP at acidic medium did not show a change until after 14 days of storage (Figure 4b), and no change in color was noted in the emulsion stabilized with WP-CMC (Figure 4c). A color change is often linked to the oxidation of polyphenolic compounds, after all, citrus fruits are rich in phenolics like the compound eriocitrin[25,26] which is responsible for pigmentation in lemons. It is obvious that the low acidic pH delays this oxidation.

As for the surface charge of the droplets, Figure 3c shows very little change in the ζ-potential. The charge of the droplet stabilized with WP-pH 6.8 and WP-CMC have increased slightly over 2 weeks but remain under −30 mV, a value at which enough

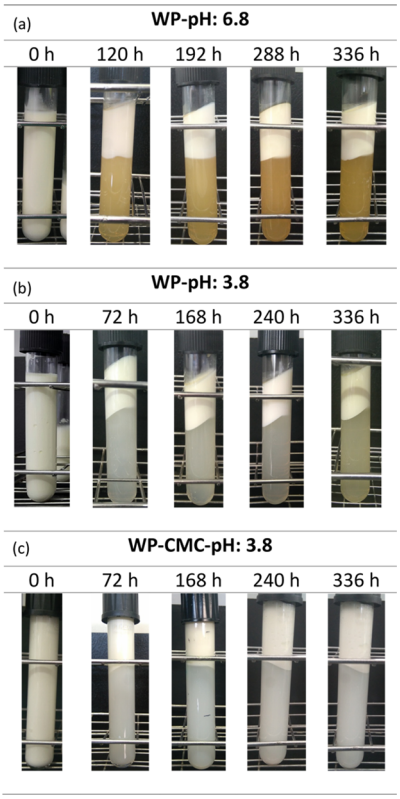

Figure 4. Physical stability of emulsions with (a) WP-pH 6.8, (b) WP-pH 3.8, and (c) WP-CMC-pH 3.8 interfaces in terms of color evolution during storage

electrostatic repulsion should ensure physical stability. In that sense, other mechanisms than electrostatic stabilization seem to control the droplet size distribution of lemon oil emulsions stabilized with either protein−polysaccharide electrostatic complexes or multilayered hydrocolloids. This is in line with what was reported for lemon oil emulsions by Almeida, Larentis, and Ferraz,[27] where the electrostatic repulsion could not be taken as a stability indicator but rather a steric repulsion. These authors found that just by mixing lemon oil (30 and 50% oil fraction) and water, and with no emulsifier, ζ-potential values of −34 and −45 mV were obtained, respectively. These values are high enough to stabilize the emulsion electrostatically, but obviously, the mixtures were not stable and separated immediately.

3.3. Stability of Volatile Compounds in Lemon O/W Emulsions Produced with SPG Membranes. To determine

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ACS Food Sci. Technol. 2022, 2, 41−48

the impact of the interfacial composition on the encapsulated aroma of lemon oil, the degradation of citral and D-limonene was followed. In particular, neral and geranial, the isomers of citral, as well as α-terpineol and carvone, the off-flavors result of the D-limonene degradation were monitored. Due to its unsaturated character, D-limonene can yield byproducts such as carvone, via radical formation by oxidative pathways. Besides, D-limonene is highly susceptible to acid-catalyzed reactions which give rise to compounds such as α-terpineol. The HS-SPME-GC method was applied to follow the compositional changes of lemon oil components in emulsions encapsulated with 1 wt % WP, pH 3.8 and 6.8, and a 0.5 wt % WP−0.25 wt % CMC, pH 3.8, electrostatic complex and stored under accelerated oxidation conditions (UV-light) for 14 days.

An example of an obtained chromatogram from analyzing the emulsion stabilized with WP-pH 6.8 is shown in Figure S1 the Supporting Information. It should also be noted that the degradation of volatile components in lemon oil, and quantified by HS-SPME-GC during storage, should be attributed to the deterioration of the emulsion as a whole, independently from the origin of deterioration. Since lemon oil is partially soluble in water deteriorating compounds could be present in the oil and/or the water phase. As explained in section 2.7.2, the evolution of the volatile compound content was monitored from the ratio of the peak area of each compound during storage to the corresponding initial peak area (A_t/A_0).

The production/release (release, i.e., no longer encapsulated) rate of α-terpineol and carvone was dramatically affected by the interfacial composition (Figure 5). Also, 1 wt % WP-stabilized

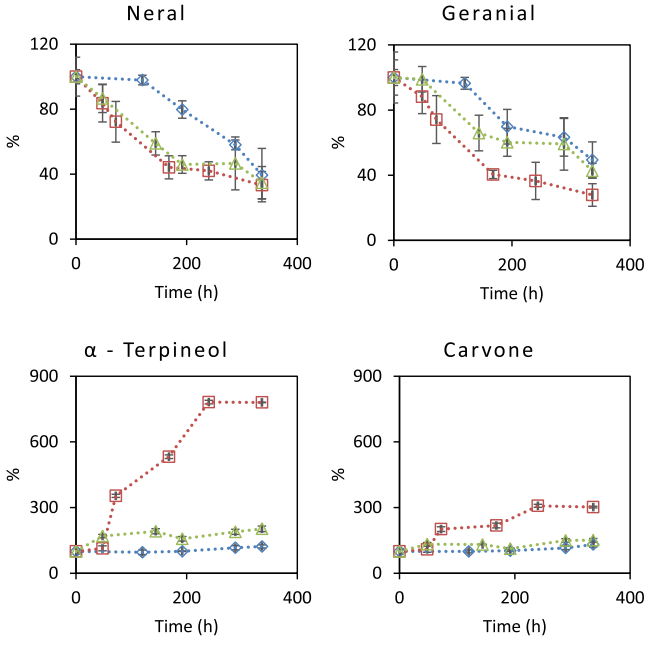

Figure 5. Progression of the percentages of compounds detected in lemon oil emulsions stabilized with (◊) WP-pH 6.8, (□) WP-pH 3.8, and (△) WP-CMC-pH 3.8, over the period of 2 weeks under UV light.

emulsions at pH 3.8 exhibited a very fast production of α-terpineol. The increase of α-terpineol was particularly abrupt as of 72 h of storage reaching an A_t/A_0 value of 300%, and by the end of the storage period it reached a value of 780%. In the same acidic conditions, pH 3.8, emulsions with WPI-CMC electrostatic complex showed a much slighter increase of A_t/A_0 from 157% to 203% after 7 and 14 days of storage, respectively. So, the production/release rate at low pH of α-terpineol, recognized as a major unpleasant off-flavor in citrus juices and result of the acidic pathway of limonene degradation, was reduced when stabilizing the oil−water interface by a 0.5 wt % WP−0.25 wt % CMC electrostatic complex. This occurred despite that the interfacial surface was negatively charged and the total WP content was reduced by 50%. For carvone, a degradation product of terpenic compounds such as D-limonene under aerobic conditions, we also observed the highest concentration in emulsions stabilized with WPI at pH 3.8 with a A_t/A_0 value of 300% over the whole storage period. Similarly to α-terpineol, the carvone produced/released was drastically reduced by using a 0.5 wt % WP−0.25 wt % CMC electrostatic complex at pH 3.8 or 1 wt % WP at pH 6.8 as stabilizers. Similar trends were reported by Sabik et al.[3] in lemon oil emulsions stabilized by Maillard conjugates of sodium caseinate and lactose.

In the case of the citral isomers, we observed a faster degradation of neral in lemon oil emulsions at pH 3.8 than in those at pH 6.8 (Figure 5), although the final A_t/A_0 (after 14 days of storage) was about 33% regardless of the emulsion pH. Results for geranial showed a fast decrease rate for WPI-stabilized emulsions at pH 3.8, which showed a final A_t/A_0 value of 28%, significantly lower than the 49% and 43% showed by emulsions stabilized with WP at pH 6.8 and WP-CMC at pH 3.8, respectively. These results would confirm a major effect of acid-catalyzed cyclization on the deterioration of these two isomers, that, for geranial, it was partially inhibited by the WP-CMC complex at pH 3.8.

It is not clear why WP at pH 3.8, where it is positively charged and can repel cationic catalysts, is behaving poorly in maintaining the chemical stability of the emulsions. Djordjevic et al.[1] made a similar observation where lower oxidation levels of citral were reported with emulsions stabilized with WP-pH 7 than with WP-pH 3. The authors explain that this might be due to the antioxidant properties in some whey protein amino acids. Also, in emulsions stabilized with subsequent interfacial coatings of whey proteins and pectin[10] (a negatively charged interface obtained by the layer-by-layer technique) a slight reduction in neral and geranial degradation was observed when compared to emulsions with a positively charged single interfacial layer of whey protein.

In light of all that, it seems that the electrical charge of the interfacial surface is not the only parameter affecting the degradation rate of citral and D-limonene, the main compounds responsible for the lemon aroma. An additional factor that may affect both the accessibility of the pro-oxidants to the interface but also how easily the already formed products of degradation are released should be considered. Interfacial physical properties such as thickness and viscosity probably play a significant role. In a previous work, Berendsen et al.,[18] it was found that the thickness of an interfacial layer made of WP was notably lower (0.6 nm) than that formed by the WPI-CMC complex (2.2 nm), both at pH 3.8. Additionally, the presence of CMC in the complex significantly increases the viscosity. Consequently, a negatively charged but thicker and more viscous interface of WPI-CMC complex would prevent the access of prooxidant cations, reducing the rate of acid-catalyzed hydration-dehydration reactions while hindering the release of the deterioration products.

In summary, this paper shows how, in lemon O/W emulsions, both the oil−water interfacial surface charge and the barrier properties can be tailored with a WP-polysaccharide electrostatic complex. With it, a thicker interfacial coating than that

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ACS Food Sci. Technol. 2022, 2, 41−48

obtained with single WP along with a negatively charged interface at acidic conditions was formed to stabilize emulsions produced by premix membrane emulsification. Although the physical stability of these emulsions in terms of droplet size distribution was not improved using the tailor-made electrostatic WP-CMC complex, other physical characteristics like emulsion color were maintained. Regarding chemical stability, the electrostatic WP-CMC complex decreased the production/release of off-flavors resulting from the deterioration of D-limonene while hindering the reduction of citral isomers under acidic and accelerated oxidation conditions.

All in all, interfacial engineering with food biopolymers (i.e., proteins and polysaccharides) together with a low energy emulsification technique able to preserve the interfacial structure, like premix membrane emulsification, seems to be an effective strategy to control the complex chemical reactions underpinning the degradation of aroma compounds in lemon oil.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsfoodscitech.1c00274.

Chromatogram showing the peaks relevant to the lemon oil compounds to be followed during the stability study as well as a table of the principal compounds present in lemon essential oil (PDF)

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Notes

The authors declare no competing financial interest.

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