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Development of Emulsions Containing L-Ascorbic Acid and α-Tocopherol Based on the Polysaccharide FucoPol: Stability Evaluation and Rheological and Texture Assessment

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Abstract: The main function of vitamin C, as an antioxidant, is to combat free radicals and prevent premature aging, smoothing wrinkles and expression lines. In addition, it acts directly on depigmentation and prevention of blemishes on the skin. In this study, natural oils (30 wt.%) and α-tocopherol (2.5 wt.%) containing oil-in-water (O/W) emulsions stabilized with the bacterial fucose-rich polysaccharide FucoPol were formulated, adding L-ascorbic acid as an antioxidant. The optimized formulations were obtained with 8.0 wt.% L-ascorbic acid for the *Olea europaea* oil formulation (C1) with a η value of 2.71 Pa.s (measured at shear rate of 2.3 s^{-1}) and E24 = 96% and with 15 wt.% L-ascorbic acid for the *Prunus amygdalus dulcis* formulation (C2) with a η value of 5.15 Pa.s (at a shear rate of 2.3 s^{-1}) and E24 = 99%. The stability of the FucoPol-based formulations was investigated over 45 days at 4 °C, 20 °C, and 30 °C. The results showed that all formulations maintained the organoleptic characteristics, with pH variations (5.7–6.8 for C1, and 5.5–6.03 for C2) within the regulations for cosmetic products ($4 \leq pH \leq 7$). The accelerated stability tests proved the formulations' stability at 4 °C with EI = 95% for C1 and EI = 100% for C2. The rheological assessment demonstrated that the formulation presents a shear-thinning and liquid-like behavior. Regarding textural parameters, formulations C1 and C2 displayed an increase in firmness and consistency with similar spreadability during the shelf life. These findings further demonstrate FucoPol's functional properties, acting as an emulsifier and stabilizer polysaccharide in cosmetic formulations containing L-ascorbic acid.

Keywords: FucoPol; O/W emulsion; L-ascorbic acid; α-tocopherol; antioxidant; rheology

Citation: Baptista, S.; Baptista, F.; Freitas, F. Development of Emulsions Containing L-Ascorbic Acid and α-Tocopherol Based on the Polysaccharide FucoPol: Stability Evaluation and Rheological and Texture Assessment. *Cosmetics* **2023**, *10*, 56. https://doi.org/10.3390/cosmetics10020056

Academic Editor: Enzo Berardesca

Received: 1 March 2023
Revised: 22 March 2023
Accepted: 23 March 2023
Published: 27 March 2023

1. Introduction

Representing 36% of the global cosmetic market, the skincare market is one of the leaders of the cosmetic industry. Product-wise, skin health enhancement and aging reduction demand by consumers have prompted the industry to include bioactive ingredients, such as vitamins (e.g., vitamin C), in their formulations [1]. The biologically active form of vitamin C, also known as ascorbic acid, has well-known positive effects on the skin [2–5], acting as an antioxidant to prevent oxidative damage (photoaging). Ascorbic acid action as an antioxidant is based on its specificity to neutralize reactive oxygen species, such as free oxygen radicals, superoxide, and hydroxyl, preventing molecular processes that lead to the skin's photoaging [4,6]. It has also been reported as a cofactor in collagen synthesis, which reduces wrinkles, and as a tyrosinase inhibitor helping to reduce hyperpigmentation [7,8]. For these reasons, this ingredient is widely used in pharmaceutical and cosmetic formulations [4,6,9].

Ascorbic acid is highly soluble in water, being easily oxidized and forming dehydroascorbic acid in its solubilized form [4,7]. Although ascorbic acid oxidation is an issue during storage, it depends on several factors such as temperature, light, pH, and storage conditions and can happen in aerobic and anaerobic conditions [3–5,7,10]. This problem may be overcome by combining ascorbic acid with α-tocopherol, a lipid-soluble antioxidant in low-density lipoprotein and lipid membrane oxidation, due to their synergistic interactions. In fact, ascorbic acid has been suggested as a regenerator of α-tocopherol from its radicals by several studies which reported an increase of antioxidant effects of α-tocopherol in the presence of ascorbic acid [4,6,11–13]. Therefore, in skin care formulations, the combined utilization of both ingredients has been suggested [4,9]. Ascorbic acid is responsible for the protection of cellular aqueous compartments and α-tocopherol protects lipid structures by inhibiting lipid peroxyl radicals' propagation [14–16]. The combination of L-ascorbic acid 15% and α-tocopherol 1.0% promoted, in comparison to stand alone formulations of each ingredient, greater protection against erythema and prevented the formation of thymine dimers formation, as reported by Lin et al. [12]. Regarding L-ascorbic acid instability in aqueous media, several derivatives have been used by the cosmetic industry, such as ascorbyl 6-palmitate, tetra-isopalmitoyl ascorbate, magnesium ascorbyl phosphate, sodium ascorbyl phosphate, ascorbyl 2-glucoside, ascorbyl 2-phosphate-6-palmitate, and 3-O-ethyl ascorbate. The necessary enzymatic conversion to free ascorbic acid in the skin assigns a relatively low topical efficiency for those derivatives [4,17]. Moreover, the introduction of ascorbic acid in cosmetic and dermatological formulations, such as O/W and W/O emulsions [5,16], results in slower degradation rates of ascorbic acid at higher pH values, compared to aqueous solutions [14], which suggests a higher protection of ascorbic acid in emulsions.

The exopolysaccharide FucoPol produced by the bacterium *Enterobacter* A47 was reported as a promising ingredient for cosmetics, given its demonstrated bioactivity. FucoPol was shown to protect Vero cells against H_2O_2-induced acute exposure [18]. Guerreiro et al. [19] also reported the photoprotective effect of FucoPol via in vitro radiation exposure experiments of adhered Vero epithelial and PM1 keratinocytic cells. It was also demonstrated that FucoPol, as well as FucoPol-based emulsified creams, promoted migration of HFFF2 and HaCaT cells [20,21]. FucoPol's capacity as an emulsifier [22–24] was also reported. Baptista et al. [22] showed FucoPol's high performance as an emulsifier for olive oil/α-tocopherol emulsions, with its presence improving the physicochemical and structural characteristics of the emulsions. FucoPol was also shown to be effective in stabilizing and emulsifying creams, contributing to a stable shelf life, resulting in biocompatible creams with physicochemical characteristics comparable to commercial cosmetic products [25].

The objective of the present study was to develop FucoPol-based emulsified formulations containing L-ascorbic acid and α-tocopherol. The formulations were prepared with either *Olea europaea* fruit (olive) oil or *Prunus amygdalus dulcis* (almond) oil and characterized in terms of their stability, as well as rheological and textural properties.

2. Materials and Methods

2.1. Materials

FucoPol was produced via the bioreactor cultivation of *Enterobacter* A47, as previously described [26], and recovered from the cell-free supernatant via ultrafiltration with a 30 kDa membrane, according to the method described by Baptista et al. [23]. The biopolymer was composed of 40 mol% fucose, 29 mol% glucose, 24 mol% galactose, and 7.0 mol% glucuronic acid, with a total acyl groups content of 11.6 wt.% [23]. The sample had protein and inorganic salts contents of 8.2 wt.% and 4.0 wt.%, respectively. *Olea europaea* fruit oil and *Prunus amygdalus dulcis* oil were purchased from a local market. α-tocopherol, L-ascorbic acid, methyl paraben, and cetyl alcohol were acquired from Sigma-Aldrich (Munich, Germany). *Olea europaea* fruit oil, composed of squalene, phytosterol, tocopherol, vitamins A and E, and fatty acids (oleic and linoleic acids), is an anti-aging ingredient indicated for dermatology applications due to its acidity, antioxidant activity, and soothing

effect [22,27]. *Prunus amygdalus dulcis* is a non-toxic, non-irritating, non-sensitising and non-comedogenic ingredient, being an easily emulsifiable and water insoluble oil with a positive spreading coefficient and a high solubility effect in lipophilic cosmetic raw materials. These penetrating, moisturising, and restructuring properties are widely appreciated in the cosmetic industry, with this oil being present in several cosmetic products [28,29]. Triethanolamine (TEA) was acquired from Acros Organics B.V.B.A. (Geel, Belgium), and glycerine was acquired from Honeywell (Seelze, Germany).

2.2. Preparation of FucoPol-Based Emulsions

FucoPol-based emulsions were prepared with either *Olea europaea* (olive) fruit oil or *Prunus amygdalus dulcis* (almond) oil, comprising different L-ascorbic acid concentrations (5, 8, 10, and 15 wt.%). The emulsions (5 g) were prepared by heating the oil phase (1.63 g), comprising either olive oil or almond oil (30 wt.%), cetyl alcohol (1.5 wt.%), and α-tocopherol (2.5 wt.%), and the aqueous phase (3.37 g), comprising FucoPol (1.5 wt.%), glycerine (3.0 wt.%), TEA (0.5 wt.%), methyl paraben (0.02 wt.%), and purified water (q.s. 100 wt.%), at 75 °C, in a recirculated Thermomix® ME water bath (B.Braun, Melsungen, Germany). L-ascorbic acid was added to the aqueous phase. The preparation of the aqueous phase involved a pH adjustment step to ~5.5 (made with TEA). The mixtures were emulsified via manual agitation for 40 s, followed by vortex agitation for 10 s. The emulsification index (EI, %) was determined using the following equation:

$$EI = \frac{h_e}{h_T} \times 100 \quad (1)$$

where h_e (mm) is the emulsion's layer height and h_T (mm) is the overall height of the mixture.

2.3. Preparation of FucoPol-Based Formulations

Two formulations, C1 and C2, with different L-ascorbic acid concentrations (8.0 and 15 wt.%, respectively) were prepared according to Table 1 (adapted from [24]). The oil phase (32.5 g) and the aqueous phase (67.5 g) were heated at 75 °C. The emulsification was performed by slowly adding the oil phase to the aqueous phase and mixing at a shear rate of about 11,000 rpm (IKA T25 easy clean digital ULTRA TURRAX, Staufen, Germany), for 3 min, followed by manual continuous agitation until room temperature was attained [30]. All formulations were prepared in batches of 100 g.

Table 1. Cosmetic formulation composition (wt.%). q.s.—quantity sufficient.

INCI Name	Function	Concentration (wt.%)	
Aqueous phase		C1	C2
Water	Solvent	q.s. 100	q.s. 100
FucoPol	Emulsifier agent	1.5	1.5
L-ascorbic acid	Antioxidant	8.0	15
Glycerine	Emollient/humectant	3.0	3.0
Methyl paraben	Preservative	0.02	0.02
TEA	pH regulator	q.s.	q.s.
Oil phase			
Cetyl alcohol	Co-emulsifier agent	1.5	1.5
Olea europaea (Olive) fruit oil	Oil, dispersed phase	30	-
Prunus amygdalus dulcis (almond) oil	Oil, dispersed phase	-	30
α-tocopherol	Antioxidant	2.5	2.5

2.4. Formulations' Characterization

The organoleptic (colour, odour) and macroscopic appearance of formulations were visually evaluated. The EI was determined during the storage period (t = 1, 3, 7, and

45 days) using Equation (1). Accelerated stability tests were performed at 4 °C and 30 °C over a period of 45 days. During the shelf life period, the samples (5 g) were stored in 10 mL closed test tubes protected from light by an aluminium foil. The pH and the conductivity values were determined after dispersing the formulation sample in deionized water (10%, *w*/*w*) [31–33]. The emulsion type was determined by placing a droplet of the test emulsion onto Whatman™ filter paper (0.2 µm, GE Healthcare Life Sciences, Munich, Germany) and observing the droplet's dispersion. For the microscopic observation, 10 µL of the sample was stained with 1% (*v*/*v*) Nile blue A (Sigma-Aldrich, Darmstadt, Germany) and observed in a Zeiss Imager D2 epifluorescence microscope (Carl Zeiss, Oberkochen, Germany), with a magnification of 40× through ZEN lite software (Carl Zeiss, Oberkochen, Germany). The physical stability was evaluated by centrifuging 1 g of the sample, at 4800 rpm, for 30 min [34]. Dynamic light scattering (DLS) was performed to determine the average particle size, the polydispersity index (PI), and the zeta potential, using a nanoPartica SZ-100V2 series (Horiba, Lier, Belgium) with a laser of 532 nm and controlling the temperature with a Peltier system (25 °C). DLS measurements were performed by diluting the samples (1:10, *w*/*w*) in a disposable cell with a scattering angle of 90°. Cumulants statistics data analysis was performed to determine the hydrodynamic size and polydispersity. Zeta potential measurements were performed in a graphite electrode cell with a 173° scattering angle [20].

2.5. Viscoelastic Properties

The formulations' rheological properties were studied using an MCR 92 modular compact rheometer (Anton Paar, Graz, Austria), equipped with a CP35-2 cone-plate sensor system (angle 2°, diameter 35 mm) and a P-PTD 200/AIR Peltier plate to keep the measurement temperature constant at 25 °C. Dynamic viscosity measurements were performed with a shear rate between 0.01 and 1000 s^{-1}. Frequency sweep analysis was performed with frequencies ranging from 0.01 to 10 Hz for a constant strain of 0.1–1.0% that was well within the linear viscoelastic limit evaluated through preliminary amplitude sweep tests.

2.6. Texture Analysis

The firmness, consistency, cohesiveness, and adhesivity of the attained formulations were determined using a texture analyser (TMS-Pro, Food Technology Corporation, Sterling, VA, USA) equipped with a 10 N load cell (Mecmesin, Sterling, VA, USA). The sample was placed in a female conic holder and was compressed to a depth of 11 mm (which represented a sample deformation of around 70%); this procedure was done twice using a male conic probe at a speed of 2 mm/s.

3. Results and Discussion

3.1. Defining L-Ascorbic Acid Concentration in the Emulsions' Continuous Phase

The FucoPol-based emulsion formulations, containing either *Olea europaea* fruit oil or *Prunus amygdalus dulcis* oil, were supplemented with different L-ascorbic acid concentrations (5, 8, 10, and 15 wt.%) to evaluate its impact on the EI at 24 h (E24) and their apparent viscosity. The resulting *Olea europaea* fruit oil containing emulsions presented a yellow colour (Figure 1, upper images) and a slight olive odour, whilst those containing *Prunus amygdalus dulcis* displayed a white colour (Figure 1, lower images) and smooth almond scent. Both formulations presented high E24 values (98–99% for *Prunus amygdalus dulcis* oil emulsions and 89–96% for *Olea europaea* fruit oil emulsions), for all tested L-ascorbic acid concentrations (Table 2). These values are identical to those reported for FucoPol-based emulsion (98%) with the same contents in *Olea europaea* fruit oil (30%) and α-tocopherol (2.5%) (formulation C) [24].

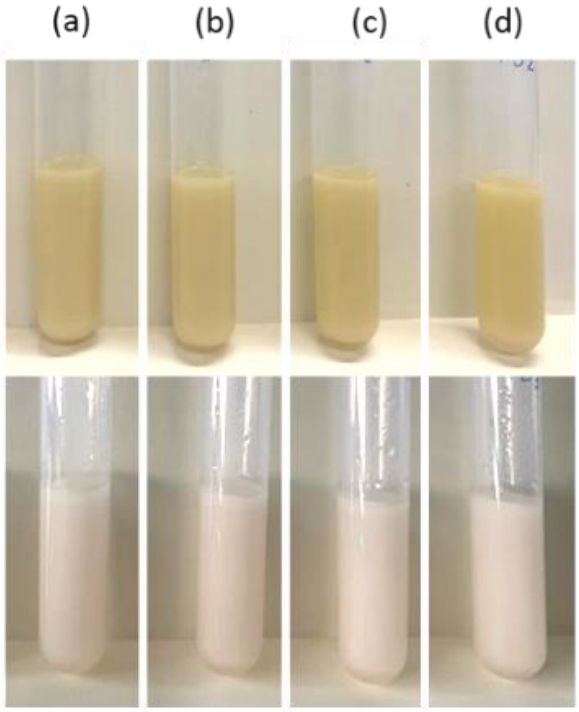

Figure 1. FucoPol-based emulsions prepared with *Olea europaea* fruit oil ((**upper**) images) and *Prunus amygdalus dulcis* oil ((**lower**) images), supplemented with different L-ascorbic acid concentrations: (**a**), 5.0 wt.%; (**b**), 8.0 wt.%; (**c**), 10 wt.%; (**d**), 15 wt.%.

Table 2. pH, conductivity, E24, and apparent viscosity (η, measured at 2.30 s^{-1}) for the emulsified formulations containing different L-ascorbic acid concentrations. Data obtained under the same manufacturing and scale conditions.

Natural Oil	L-Ascorbic Acid (wt.%)	E24 (%)	η (Pa.s)	References
Olea europaea	5	89 ± 0.0	3.33	This study
	8	96 ± 0.0	2.71	
	10	96 ± 0.0	2.52	
	15	96 ± 0.0	1.92	
Prunus amygdalus dulcis	5	98 ± 0.0	3.23	This study
	8	98 ± 0.0	3.82	
	10	98 ± 0.0	4.59	
	15	99 ± 0.0	5.15	
Olea europaea (Formulation C)	-	98 ± 0.0	28.1	[24]

All emulsions exhibited a shear thinning behaviour to the torque response (Figure 2) characteristic of FucoPol-based formulations [24] as the viscosity gradually decreased under increasing shear rates [35]. Nonetheless, supplementing the formulations with L-ascorbic acid resulted in a significant decrease of the samples' apparent viscosity (1.92–5.15 Pa.s, measured at a shear rate of 2.30 s^{-1}) (Table 2), compared to FucoPol-based formulation C, which presented an apparent viscosity of 28.1 Pa.s [24]. Moreover, the emulsions containing *Olea europaea* oil displayed a slight decrease of the apparent viscosity, from 3.33 Pa.s to 1.92 Pa.s for increasing L-ascorbic acid concentrations (from 5 to 15 wt.%, respectively), while those containing *Prunus amygdalus dulcis* oil had the opposite trend, with the apparent viscosity gradually increasing from 3.23 Pa.s to 5.15 Pa.s. These results are probably related to the fatty acid composition of each oil. *Olea europaea* and *Prunus amygdalus dulcis* oils are both mainly composed of oleic acid (65–80%) but differ in their contents of palmitic and linoleic acids. *Prunus amygdalus dulcis* oil was richer in palmitic acid (9.3–12.5%), a

saturated C16 fatty acid, than *Olea europaea* oil (6–7%) [36–38]. Further, *Prunus amygdalus dulcis* oil contains linolenic acid (0.42–1.91%), an unsaturated C18 fatty acid, which is not present in *Olea europaea* oil. These differences in carbon content and structure of the oils' fatty acids are likely to result in different interactions with L-ascorbic. Carrillo et al. [39] reported such factors to be decisive in determining an emulsion's rheological behaviour.

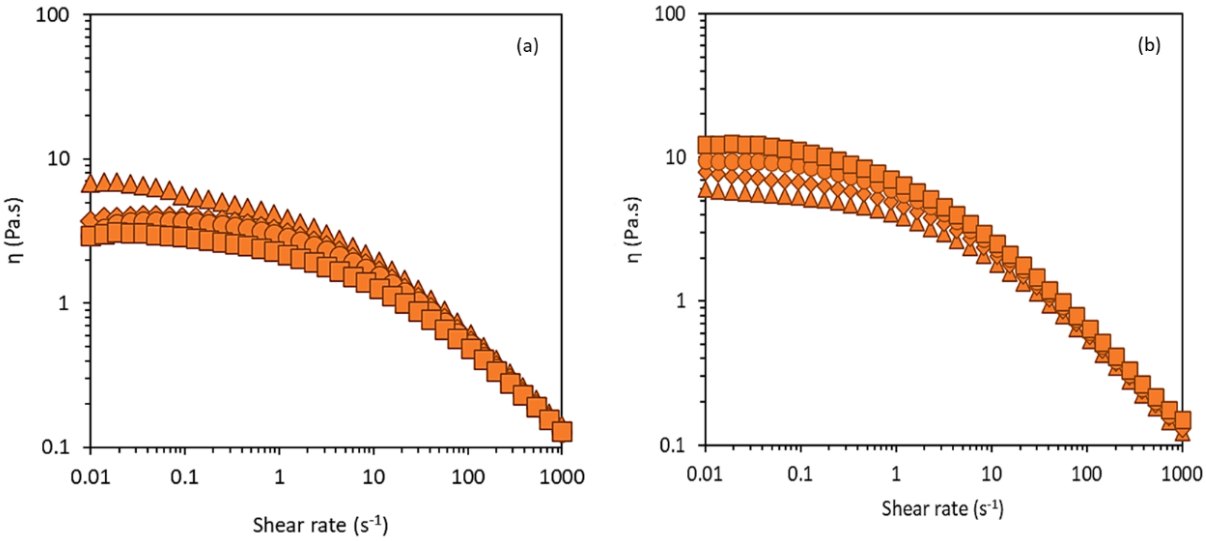

Figure 2. Flow curves for the FucoPol-based emulsions (analysed after 24 h of preparation) with (**a**) *Olea europaea* fruit oil and (**b**) *Prunus amygdalus dulcis* oil, supplemented with L-ascorbic acid at concentrations of: 5.0 wt.% (triangles), 8.0 wt.% (diamonds), 10 wt.% (circles), and 15 wt.% (squares).

3.2. Emulsified Formulations with L-Ascorbic Acid

Two formulations (named C1 and C2) were designed based on the FucoPol-based cream previously developed by Baptista and Freitas [24]. The L-ascorbic acid concentration for each formulation was chosen considering the results obtained for *Olea europaea* and *Prunus amygdalus dulcis* oils in terms of apparent viscosity and E24 value. Thus, the L-ascorbic acid concentration chosen for the *Olea europaea* oil was 8.0 wt.% with a η value of 2.71 Pa.s (measured at a shear rate of 2.3 s^{-1}) and E24 = 96% and 15 wt.% for *Prunus amygdalus dulcis* with a η value of 5.15 Pa.s (at a shear rate of 2.3 s^{-1}) and E24 = 99%. For cosmetic formulations containing ascorbic acid, the optimal concentration for a required effect must be higher than 5.0 wt.% [4,40], which was also a decision factor.

3.3. Characterization of FucoPol-Based Emulsified Formulations

3.3.1. Organoleptic Characteristics and Physical Stability

Regarding the macroscopic observation of the freshly prepared formulations (Figure 3a), formulation C1 presented a yellowish white colour and a slight olive oil odour characteristic of the previously formulated FucoPol-based formulation C [23]. Formulation C2 presented a bright white colour and slight marzipan odour due to the presence of almond oil. Throughout the 7-day storage period, the formulations maintained their homogeneous texture, with no visible oil/water phase separation, also confirmed by their unchanged EI (100%) (Figure 4a). However, after 45 days of storage, both formulations lost stability, evidenced by a decrease of the EI to 90% for formulation C1 and to 93% for formulation C2. Nevertheless, the formulations maintained the initial colour during the storage period, indicating no detectable L-ascorbic acid degradation had occurred [5,15,41]. The formulations lower stability compared to the previously reported formulation C [24] was also evidenced by their creaming after 24 h, as shown via the centrifugation test (Figure 4b). This breaking mechanism, often observed in skin lotion emulsions [42], results from centrifugal external forces exceeding the Brownian motion of droplets, creating a concentration gradient with low density droplets shifting to the top [43,44]. These results indicate that the addition of

L-ascorbic decreased the physical stability of FucoPol-based emulsion, even using *Prunus amygdalus dulcis* oil instead of *Olea europaea* oil, compared to formulation C [24].

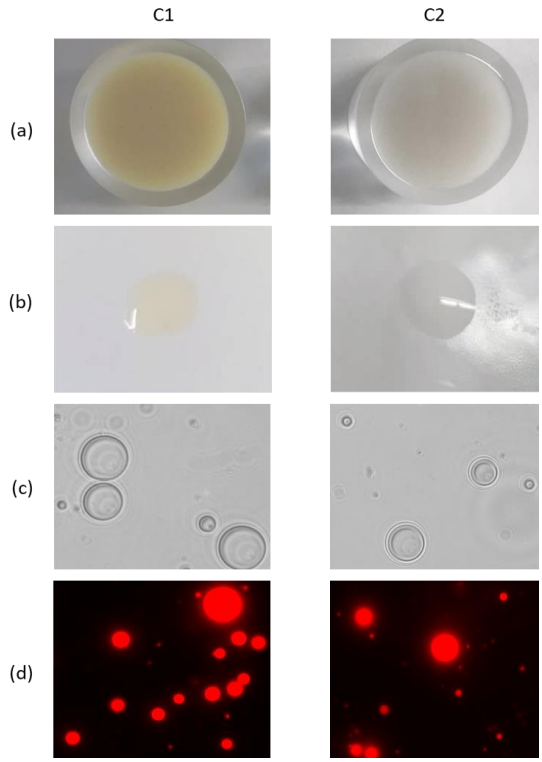

Figure 3. Formulations C1 and C2 (**a**); emulsion determination test using filter paper wetting (**b**); optical microscopic (100×) observation under contrast phase (**c**) and fluorescence after Nile blue A staining (**d**).

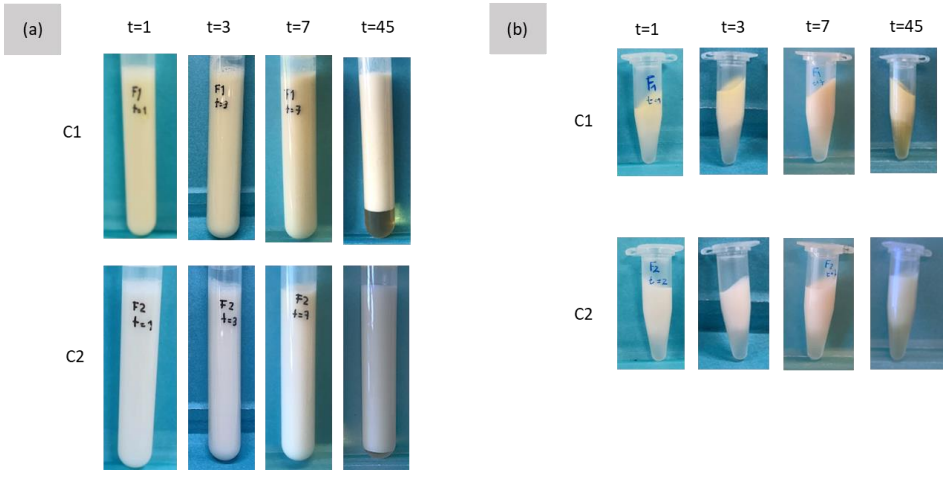

Figure 4. (**a**) Emulsification index over storage period (t = 1, 3, 7, 45 days); (**b**) centrifugation test over storage period (t = 1, 3, 7, 45 days).

3.3.2. Emulsion Type

The emulsions' droplets rapidly dispersed on the filter paper (Figure 3b), confirming their O/W nature [45,46], similar to previously reported FucoPol-based formulations [22,24]. Furthermore, upon microscopic observation (Figure 3c,d), compartmentalized structures characteristic of O/W emulsions, consisting of dispersed oil droplets in the aqueous phase [47,48], were observed, thus confirming the formulations' O/W nature.

3.3.3. pH and Conductivity

The pH and conductivity values over the storage period are represented in Figure 5a. The pH values for formulations C1 and C2 were slightly acidic (5.7 and 5.5, respectively) which were maintained during the 7-day storage period (Figure 5a). After 45 days, the pH value increased for both formulations C1 and C2 (6.8 and 6.03, respectively), but remained within the optimal range (4.0–7.0) [49–51] reported to avoid skin irritation [52], thus supporting their suitability for topical use. Additionally, the pH stability during the storage period (45 days) indicated that both emulsified systems provided protection from ascorbic acid, because a decrease in pH would be indicative of vitamin C degradation [14,53]. The oxidation process leads to the release of hydrogen ions from the enediol group of L-ascorbic acid, ultimately resulting in the decline of pH values [53], which was not observed for either formulation. Additionally, Mosca et al. [54] reported the prevention of oxidative damage in the oil phase with the addition of L-ascorbic acid in the water phase.

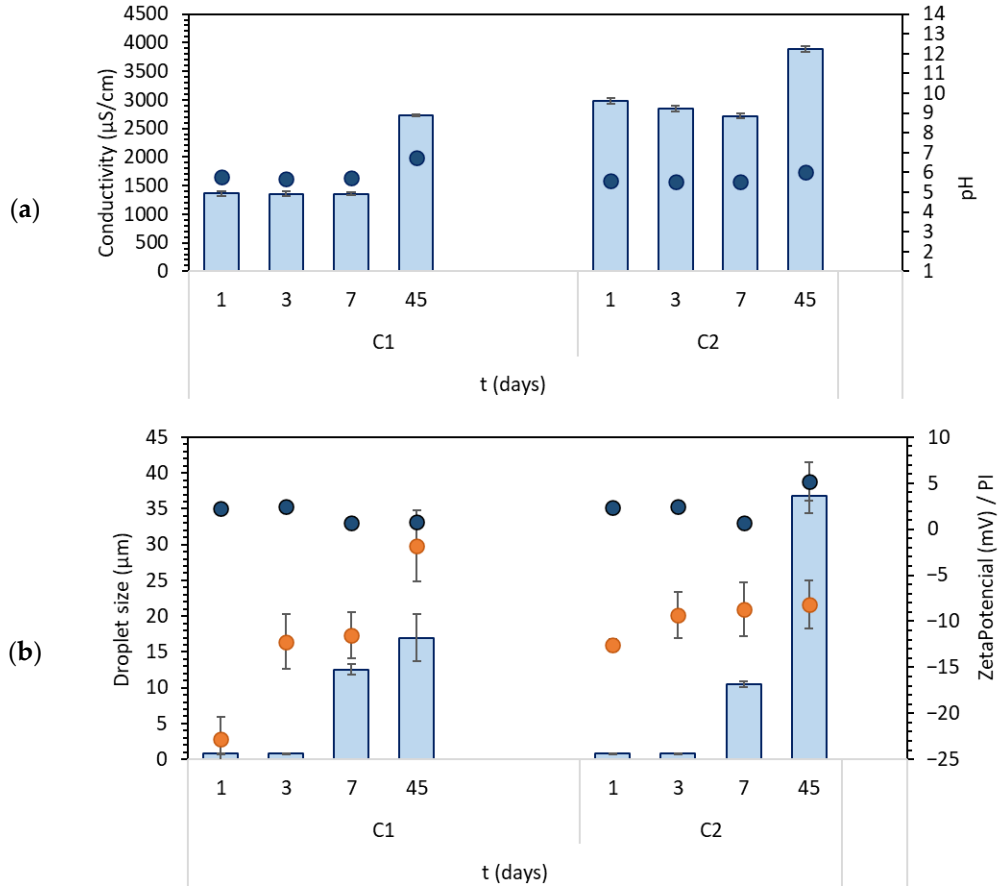

Figure 5. (**a**) pH (dots) and conductivity (bars) and (**b**) droplet size (blue bars), zeta potential (orange dot), and PI (blue dot) for formulations C1 and C2 during the storage period (t = 1, 3, 7, 45 days).

The conductivity value, which is indicative of the free ions and water content in the system [50], can be used to detect physical modifications [55] and to assess if the formed emulsion is an O/W or a W/O system [32,51]. Formulations C1 and C2 showed high conductivity values (>1300 µm/cm) characteristic of O/W systems [51], corroborating the results obtained by the emulsion determination test and the microscopic observation. As observed in Figure 5a, formulation C1 maintained the conductivity values during the 7-day storage period (1354–1360 µS/cm), increasing to 2728 µs/cm after 45 days, while formulation C2 showed a minor decrease in the conductivity value (from 2986 to 2714 µS/cm) during the 7-day storage period, and an increase to 3893 µs/cm after 45 days. The behaviour of formulation C1 was similar to formulation C [24], maintaining the conductivity

values (110–138 µS/cm), despite the increase of free ions in the system that can be correlated to the presence of L-ascorbic acid. Conductivity change over the 45-day storage period suggests the occurrence of physical alterations, which are usually related to the increase of oil proportion in the upper part of the emulsion and water proportion increase in the lower part of the emulsion, leading to emulsion creaming/sedimentation [51].

3.3.4. Droplet Size and Zeta Potential

The physical stability of formulations C1 and C2 was also assessed by measuring the droplet size during the storage period at room temperature (~20 °C). The distribution profile of oil droplets and their size influence the emulsions' stability, with smaller droplet sizes and lower PI values (<0.3) being correlated with higher stability of emulsions [30,50,56–58]. As shown in Figure 5b, all formulations presented a droplet size characteristic of macroemulsions (>0.1–50 µm) [59], experiencing a considerable increase in droplet size after 45 days of storage. For C1, the droplet size increased from 0.8 µm to 17 µm, while for C2 it increased from 0.8 to 37 µm. The droplet size of an emulsion is determined by the homogenization technique applied, the environmental conditions, and the ingredients used for its preparation [60]. Moreover, the movement of dispersed droplets through the continuous phase increases the probability of droplet collisions, resulting in higher droplet sizes [61]. Emulsion droplet production is an energy-demanding step, especially in rotational speed dependent processes, such as rotor-stator homogenizations, in which higher applied energies result in the production of smaller droplets. Moreover, additional energy is required to ensure a uniform droplet size distribution [10].

Comparing formulations C1 and C2 to the FucoPol-based emulsion, formulation C [24], the addition of L-ascorbic acid leads to an 87% decrease in droplet size. A small droplet size usually improves the stability of emulsions by favouring Brownian motion over gravity, preventing creaming/sedimentation mechanisms regardless of the storage period. Additionally, having a small droplet size contributes to flocculation and coalescence inhibition [62]. However, contrary to what was expected, formulations C1 and C2 showed creaming after 1 day of storage, while formulation C showed a breaking mechanism (phase separation) after 30 days of storage.

The ideal monodisperse system should have a PI value lower than 0.3 [32,57], which was not verified for either C1 or C2 ($0.65 \leq PI \leq 5.2$ for t = 45 days), indicating considerable polydisperse droplet sizes. However, while in formulation C the PI remained constant during the storage (between 2.2–2.9), in C1 a significant decrease of the PI was noticed between days 3 and 45 (2.4 and 0.77, respectively), while for formulation C2 a significant increase was observed between days 7 and 45 (0.66 and 5.2, respectively).

As shown in Figure 5b, the zeta potential for formulations C1 and C2 was −22.0 mV and −12.6 mV, respectively. A formulation is considered stable when the zeta potential value is above +25 mV or below −25 mV [50]. Although for C1 the zeta potential value was close to −25 mV, during the storage period, both C1 and C2 suffered a decrease of the zeta potential values, corroborating their observed lower stability. L-ascorbic acid has been reported to adsorb at the oil–water interface and promote droplet fusion, leading to their separation from the emulsion oil layer, causing instability of the entire system [63]. Formulation C presented higher absolute zeta potential (−98 mV) and pH (6.3) values. L-ascorbic acid decreases the pH of the formulation, and it was previously reported [64] that a higher pH promotes a higher zeta potential, increasing the interface's repulsive force. Considering the structure of an O/W system, with water being a high dielectric fluid and oil being a low dielectric fluid, the repulsive force between charged droplets from the interface promotes the stabilization of the emulsion, due to the asymmetric distribution of counter-ions at the interface, creating a dipole normal to the fluid–fluid interface [65]. Lower pH values cause the neutralization of the interface charges which promotes the gradual separation of surfactant from the O/W interface, leading to lower emulsion stability [64].

3.3.5. Apparent Viscosity and Viscoelastic Properties

Formulations C1 and C2 presented a shear-thinning behaviour (Figure 6), as the viscosity progressively decreased under increasing shear rates, in agreement with previous results for FucoPol-containing emulsions [22,23]. This pseudoplastic behaviour is ideal for cosmetic formulations as it promotes the sliding of the formulation on the skin, creating a protective layer and acting as a shear condensing fluid [66]. Up to day 3, formulation C1 (Figure 6a) showed lower apparent viscosity than formulation C2 (Figure 6b). Considering that *Olea europaea* oil (83.5 Pa.s) has a higher viscosity than *Prunus amygdalus dulcis* oil (56.8 Pa.s) [67], the higher L-ascorbic acid concentration used in C2 (15 wt.%) may have led to a viscosity increase. L-ascorbic may increase water phase viscosity, leading to a decrease in the droplets' mobility and collision numbers, which can explain the observed behaviour.

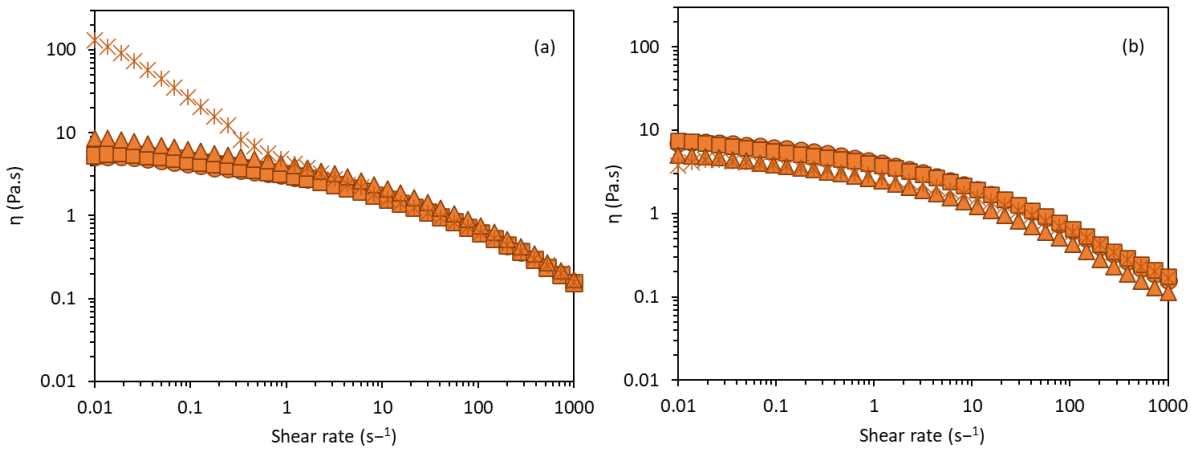

Figure 6. Flow curves for C1 (**a**) and C2 (**b**) formulations during the storage times: t = 1 (circles), t = 3 (squares), t = 7 (triangles), and t = 45 (asterisk).

The apparent viscosity at a given shear rate is also an important parameter to predict the behaviour of the cosmetic formulation in different physical operations: squeezing (0.1 s^{-1}), pouring (10 s^{-1}), and rubbing (100 s^{-1}) [66]. Hence, formulation C2 (6.9 Pa.s) seems to be more squeezable than formulation C1 (5.2 Pa.s). On the other hand, both formulations C1 and C2 have analogous values for pouring (1.65 Pa.s and 1.92 Pa.s, respectively) and rubbing out (0.62 Pa.s and 0.61 Pa.s, respectively).

The mechanical spectra of the FucoPol-based formulations are illustrated in Figure 7. Both formulations C1 and C2 showed liquid-like behaviour, with the loss module storage module higher than the storage module ($G'' > G'$ at 0.1 Hz) [23]. While for C1 the G'' and G' modules showed similar profiles during the storage period (Figure 7a); for C2 the G'' and G' modules showed higher values at 1 day and decreased thereafter (Figure 7b). This behaviour indicated the lower stability of this formulation, the dominance of the viscous components over the elastic components, and that the physical bonds between the macromolecules do not have the capacity to maintain the system's structure [68]. This behaviour contrasts with that of the FucoPol-based emulsion formulation C [24], where the storage module was higher than the loss module ($G' > G''$ at 0.1 Hz), showing a gel-like behaviour characteristic of cosmetic formulations [30,69,70]. In both systems (Figure 7), it was possible to observe that G' and G'' increase with an increase in frequency, indicating that G' and G'' are functionally dependent on frequency, implying a less stable system, corroborated by the observed breaking mechanism (creaming) [64] after the centrifuge test (Figure 4b). Contrarily, a previously reported formulation C [24] presented an almost constant G' and G'', indicating a more stable system.

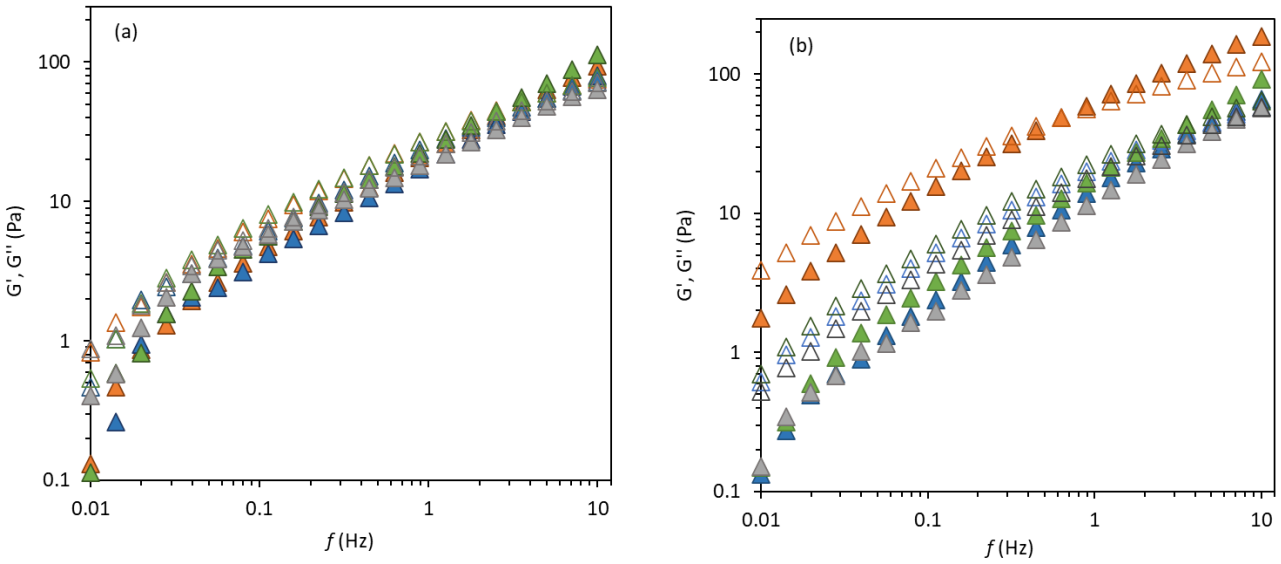

Figure 7. Mechanical spectrum for formulations C1 (**a**) and C2 (**b**) during the storage time: t = 1 (orange), t = 3 (blue), t = 7 (green), and t = 45 (grey). G′ (closed triangle) and G″ (open triangle).

Crossover frequency is an indicator of a material's viscoelastic behaviour, in which lower crossover values translate into more elastic materials [71,72]. For formulation C1, the crossover was maintained at 2.5 Hz after 3 days of storage and increased to 5.1 Hz after 7 days, disappearing after 45 days. For formulation C2 a shift was observed of the crossover value (from 0.6 to 10 Hz) during the storage period. The initial crossover value for C2 indicated the formation of a more structured formulation, which declined over the storage period. This observed shift of the crossover point towards higher frequencies over time can be attributed to the ageing of formulation C2, yielding a wider plateau region arising from the increased biopolymer molecules' entanglement density and from higher oil droplet packing [73]. This effect could be observed in the creaming mechanism from Figure 4b, which was more evident in formulation C2 than in C1. For formulation C1, the loss of structure was not so pronounced, displaying a crossover point at high frequency after 1 day of storage. The results obtained suggest that when an emulsion presents a wider plateau relaxation zone after a short ageing time, it often translates into longer physical stability [73]. In summary, formulation C2 was initially the most structured system and storage time affecting (declined) this characteristic; formulation C1 started as the less structured system but its structure loss was not as pronounced as in formulation C2.

The pH value can also influence the emulsion's flow behaviour. The lower pH values of formulations C1 and C2 (5.7 and 5.5, respectively), compared to formulation C (pH = 6.3), apparently decreased the values of G′ and G″, leading to a more viscous fluid (rather than elastic), which might explain the lower stability of emulsions C1 and C2 [64,74].

3.3.6. Textural Assessment

The textural parameter values (firmness, consistency, cohesiveness, and adhesiveness) of the prepared formulations are summarized in Table 3. At the end of storage time (t = 45 days), an increase in firmness and consistency of the formulations was observed, indicating that C1 was slightly less spreadable (usually attributed to higher firmness and consistency values) than C2. The spreadability, or the skin cover capacity over time, is crucial in cosmetic emulsion development and is a decisive factor for consumers' approval of products [75]. Furthermore, at shear rates that represent the skin spreading process (1000 s^{-1}) [75], both C1 and C2 emulsions displayed similar viscosity (0.16 Pa.s). While both samples showed lower adhesivity, emulsion C1 (0.077 mJ) seemed to be more adhesive than C2 (0.073 mJ). Therefore, cohesiveness was also an important parameter to be observed, and formulation C2 was slightly more cohesive than C1. These results once again prove the

negative effect of the addition of L-ascorbic acid. Compared to the FucoPol-based cream C [24], L-ascorbic acid induced a decrease of 80% in firmness, 83% in consistency, and 80% in adhesiveness, after 1 day.

Table 3. Numerical values of the textural parameters for formulations C1 and C2 during the storage period at room temperature. Data obtained under the same manufacturing and scale conditions.

Formulation	Time (Days)	Textural Parameters				References
		Firmness (N)	Consistency (mJ)	Cohesiveness (N)	Adhesiveness (mJ)	
C1	1	0.039	0.065	1.027	0.077	This study
	3	0.071	0.078	0.759	0.063	
	7	0.064	0.177	0.817	0.075	
	45	0.086	0.186	0.949	0.038	
C2	1	0.039	0.053	1.069	0.073	This study
	3	0.064	0.099	0.950	0.078	
	7	0.064	0.129	1.377	0.079	
	45	0.077	0.153	0.924	0.057	
C	1	0.194	0.385	1.035	0.387	[24]
	60	0.047	0.160	1.004	0.129	

3.3.7. Accelerated Stability

The accelerated stability is an important parameter to assess the formulation behaviour during storage. The formulations were preserved for 45 days at 4 °C and 30 °C. Concerning the macroscopic organoleptic characteristics (Figure 8a), formulations C1 and C2 maintained the colour, odour, and texture regardless of storage temperature, indicating the absence of L-ascorbic acid degradation [15]. Regarding EI values (Table 4.), formulations stored at 4 °C presented higher values (95% for C1 and 100% for C2) than those stored at 30 °C (81.3% for C1 and 94% for C2) and at 20 °C (90% for C1 and 93% for C2). At 4 °C, the resistance to structural breakdown seemed to be higher, resulting in less creamed emulsions (Figure 8b). These results were corroborated with higher zeta potential values for formulation C1 (−21 mV) and C2 (−18 mV), at 4 °C. At 30 °C, C1 had −2.6 mV and C2 had −3.1 mV of zeta potential, whilst C1 and C2 had, respectively, −1.8 mV and −8.2 mV, at 20 °C. This variation of zeta potential values might be explained by chemical alterations (emulsifiers decomposition) or the development of charged molecules [61]. Zeta potentials >+25 or <−25 provide a momentary stability of the emulsion, tending to droplet gathering (flocculation and coalescence effect) [61] during storage.

The pH values for all temperatures remained similar (6.0–6.8) at the end of the storage period. At 4 °C there was higher stability during the shelf life for both formulations C1 and C2, considering the minimal difference between conductivity values obtained after 24 h and 45 days of storage (C1: 1360 to 1593 µs/cm; C2: 2986 to 2561 µs/cm). Regardless of temperature, all formulations presented non-Newtonian (shear-thinning) and liquid-like behaviour (Table 4 and Figure 9). Formulation C1, stored at room temperature (20 °C), presented higher apparent viscosity values (3.2 Pa.s, at 2.30 s^{-1}) compared to other temperatures. These fluctuations are possibly due to structural rearrangements [30]. The viscosity of cosmetic formulations containing polymers are susceptible to dry and/or humid storage conditions, influenced by temperature [42,76]. Furthermore, the increase of storage temperature promotes the decrease in viscosity, and vice-versa, resulting in a destabilization effect [61]. Emulsion viscosity can be influenced by oil phase crystallization, controlled by temperature and storage time, which induces the emulsion droplets' partial coalescence or results in conformational alterations on the biopolymer [61,77]. The temperature also appears to influence droplet size, being different for each type of formulation. Larger droplets were observed for formulation C1 at 4 °C and 30 °C (from 17 µm to 150 µm and 90.5 µm, respectively). The droplet size increase might be attributed to the coalescence of

droplets [61]. On the other hand, for formulation C2, a decrease in the droplet size was noted, when stored at 4 °C and 30 °C (from 38.8 µm to 11.8 µm and 8.97 µm). Regarding the texture parameters, the most accentuated difference is the drastic decrease in consistency for formulation C2 at 30 °C (0.021 mJ) compared to the value for 4 °C (0.205 mJ) and 20 °C (0.153 mJ). The decrease in cohesiveness at 4 °C (0.789 mJ) in the C1 formulation compared to the value at 30 °C (0.979 mJ) and 20 °C (0.949 mJ) also denotes an effect of temperature on the textural properties of the formulation.

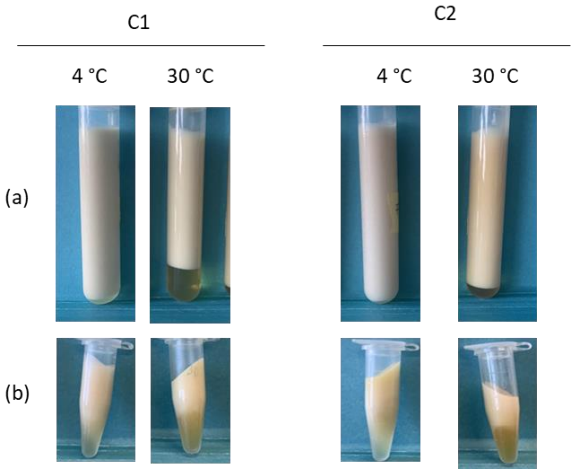

Figure 8. (**a**) Emulsification index over storage temperature after 45 days; (**b**) centrifugation test over storage temperature after 45 days.

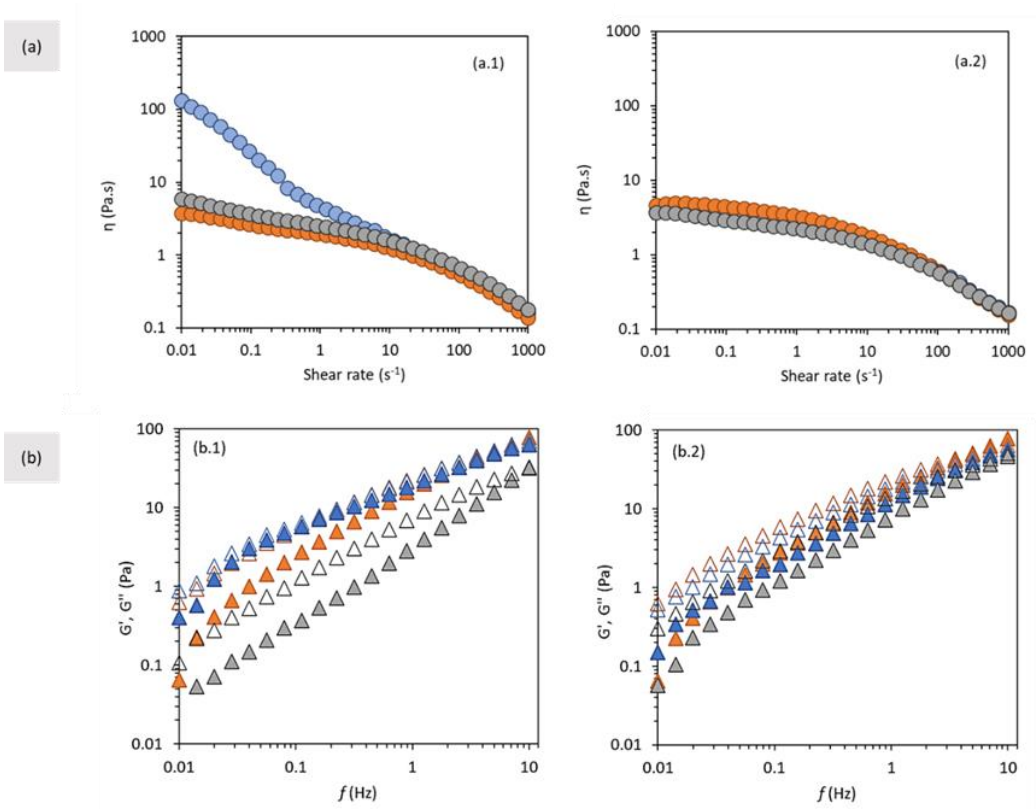

Figure 9. Rheological profile analysis of formulations C1 (**a.1**,**b.1**) and C2 (**a.2**,**b.2**): (**a**) viscosity curves as a function of the shear rate (circle); (**b**) mechanical spectra (triangle): elastic G′ (open) and viscous G″ (closed) moduli in the function of frequency; 4 °C (orange), 20 °C (blue), 30 °C (grey).

Table 4. Physicochemical characterization of formulation C1 and C2 during 45 days of storage at 4 °C, and 30 °C. η at 2.30 s^{-1}.

Temperature (°C)	Formulation	pH	Conductivity (µs/cm)	Droplet Size (µm)	EI (%)	η (Pa.s)	Firmness (N)	Consistency (mJ)	Cohesiveness (N)	Adhesiveness (mJ)
4	C1	6.6	1593 ± 12	150 ± 7.7	95	1.7	0.100	0.198	0.784	0.062
	C2	6.1	2561 ± 45	11.8 ± 0.5	100	2.8	0.065	0.205	0.914	0.083
30	C1	6.6	1596 ± 7.1	90.5 ± 3.2	81	2.2	0.099	0.200	0.979	0.043
	C2	6.4	2563 ± 29	8.97 ± 0.4	94	1.9	0.080	0.021	0.833	0.054
20	C1	6.8	2728 ± 12	17.0 ± 3.3	90	3.2	0.086	0.186	0.949	0.038
	C2	6.0	3893 ± 51	36.8 ± 2.4	93	2.4	0.077	0.153	0.924	0.057

4. Conclusions

In this study, FucoPol-based creams containing either *Prunus amygdalus dulcis* oil or *Olea europaea* oil, supplemented with α-tocopherol (vitamin E) and L-ascorbic acid (vitamin C) as bioactive agents were studied. Vitamin C has been widely used in cosmetic and dermatological products due to its physiological effects on the skin. However, it presents handling issues, inherent to its high risk of oxidation in light, at high temperatures and in the presence of water. Although resulting in particle size reduction, the presence of L-ascorbic acid in the aqueous phase resulted in O/W systems with lower physical stability, as demonstrated by the decrease of the emulsions' apparent viscosity, firmness, cohesiveness, and adhesivity, as well viscoelastic behaviour change. Macroscopic evaluation of formulations during the storage period showed no visible signs of L-ascorbic acid degradation, supported by the maintenance of their colour and odour, which is an interesting result considering the known challenges of including vitamin C in cosmetic emulsions. Although the L-ascorbic acid effect on the FucoPol-based cream resulted in a less stable formulation, it can be used for other types of cosmetics (e.g., serums).

Author Contributions: Conceptualization, S.B. and F.F.; methodology, S.B.; investigation, S.B. and F.B.; writing—original draft preparation, S.B.; writing—review and editing, F.F.; supervision, F.F.; funding acquisition, F.F. All authors have read and agreed to the published version of the manuscript.

Funding: This work is financed by national funds from FCT (Fundação para a Ciência e a Tecnologia), I.P., in the scope of the project UIDP/04378/2020, UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences (UCIBIO), and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy (i4HB).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available upon request.

Conflicts of Interest: Sílvia Baptista is employed by the company 73100 Lda. The remaining authors declare no conflict of interest.

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