

# Potential of lignin multifunctionality for a sustainable skincare: Impact of emulsification process parameters and oil-phase on the characteristics of O/W Pickering emulsions

Oihana Gordobil<sup>a,\*</sup>, Nicole Blažević<sup>a,b</sup>, Marjana Simonič<sup>b</sup>, Anna Sandak<sup>a,c</sup>

<sup>a</sup> InnoRenew CoE, Livade 6A, 6310 Izola, Slovenia

<sup>b</sup> University of Maribor, Faculty of Chemistry and Chemical Engineering, Smetanova 17, 2000 Maribor, Slovenia

<sup>c</sup> University of Primorska, Faculty of Mathematics, Natural Sciences and Information Technologies, Glagoljaska 8, 6000 Koper, Slovenia

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## ABSTRACT

Colloidal lignin particles (CLPs) from softwood kraft lignin were evaluated as a multifunctional ingredient to prepare bio-based oil-in-water (O/W) Pickering emulsions. After a preliminary screening, three Pickering emulsions systems were formulated using orange, coconut, and paraffin oils, at varying concentration of CLPs, oil/water ratio, and by applying two-step homogenisation processes (rotor-stator homogenisation followed by ultrasonication). Ultrasonication as a second homogenisation step considerably enhanced the emulsification efficiency, generating emulsions with smaller droplet size and less polydisperse distribution. Furthermore, the effect of ultrasonication on the characteristics of emulsions and the stability of the systems was evaluated over time. The oil content and type, the concentration of CLPs, and the homogenisation methods significantly influenced the characteristics of the emulsions and drop size. Higher concentrations of oil and CLPs favoured the formation of the emulsion and contributed to higher physical stability after 120 days of monitoring. The *in vitro* Sun Protection Factor (SPF) results demonstrated that the incorporation of natural oils containing phenolic compounds in Pickering formulations improves the SPF value of emulsions, showing an interesting synergic effect between lignin particles and vegetable oils, which was not observed in the case of paraffin oil. Furthermore, the broad-spectrum sun blocker of Pickering emulsions was confirmed by the values of UVA/UVB between 0.74 and 0.90 and  $C_{\lambda} > 380$ .

## 1. Introduction

The skin is our largest organ and one of the most important, acting as a protective barrier against harmful agents such as pathogens or effects of UV radiation, helping thermoregulation functions of the body and permitting sensations of touch, heat, and cold [1].

Throughout history, countless cosmetic products aimed at beautifying, purifying, cleansing, and protecting the skin have been designed [2]. Skin cosmetics, like topical formulations, are usually water-based products containing many other chemical ingredients to provide specific functionalities into the formulation such as emulsifiers, skin conditioning agents, antioxidants, preservatives, UV filters and fragrances [3].

Today, the safety profiles of certain chemical ingredients used in personal care products are being questioned due to their detrimental

toxicological effects on the ecosystems and human health. Concern about these emerging contaminants stems from numerous recent studies that have proven not only the widespread presence of many of the ingredients from these chemicals (*i.e.*, surfactants, preservatives, and organic UV filters) in the environment, but also their endocrine-disrupting effects, and tendency to bioaccumulate and bioconcentrate in living organisms [4–6].

Consequently, not only is there a growing demand from consumers for chemical-free cosmetics but also from authorities to move towards sustainable raw materials to minimise the impact of cosmetic products on biodiversity. Therefore, the exploration of safe and sustainable natural alternatives is crucial. Several research studies have demonstrated the potential of lignocellulosic components, from side streams such as polysaccharides (*i.e.*, cellulose [7], hemicellulose [8], and starch [9]) and polyphenols [10], to replace fossil-based ingredients, thus, enabling

\* Corresponding author.

E-mail address: [oihana.gordobil@innorenew.eu](mailto:oihana.gordobil@innorenew.eu) (O. Gordobil).

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the transition into a bioeconomy.

Lignin is the only high-molecular-weight natural polymer with an aromatic structure and is the most abundant polyphenolic compound present in nature. Although there are several types of lignin in terms of chemical structure and molecular weight, depending on the origin and extraction processes, Kraft lignin represents the largest source of lignin since it is present in the black liquor which is generated as a side stream during cellulose pulp manufacturing in the papermaking process, which is usually burned for energy [11].

In addition to its industrial availability, its structural features and diverse functional properties make it especially attractive for evaluation as a bio-active alternative for cosmetics and personal care products. Lignin is an antioxidant compound and capable of absorbing UV radiation due to its chemical structure, which contains a wide variety of functional groups [12]. Moreover, the antimicrobial activity of lignin could be beneficial in increasing the life period of the cosmetic product by reducing the microbial growth in the formulation [13,14]. In addition, several studies have suggested that it is a biocompatible compound and non-toxic for human health care applications at determined concentrations [15,16].

However, the application of this bio-sourced compound in high-performance applications in skincare cosmetics is hampered by its dark color, low solubility in water, and low sunscreen performance. Recent works have shown that lignin color, sunscreen performance, and other functional properties are highly influenced by particle size and morphology [17–19]. Colloidal lignin particles (CLPs) present additional advantages for cosmetic applications such as increased dispersibility in water and improved functional properties. Furthermore, CLPs can be used to stabilise immiscible liquid phases, like water and oil, by adsorption at the oil-water interface, forming the so-called Pickering-emulsions [20], which have recently garnered high interest for cosmetics since they display a longer lifetime than surfactant-based emulsions. Also, much lower concentrations of Pickering particles are required than traditional surfactants for stabilising emulsions, leading also to a considerable cost saving [21]. Moreover, Pickering emulsions stabilized by solid particles usually have higher resistance to coalescence.

Several inorganic and organic particles have been widely studied as emulsion stabilisers for topical applications [22]. Although inorganic particles like silica or titanium dioxide can be biocompatible and provide good stability, they are not biodegradable and might penetrate the skin and accumulate in the human body inducing adverse effects [23]. Therefore, particles from biomass based on natural polymers such as starch, cellulose, chitin, and lignin are considered as very promising sustainable solutions for the stabilisation of emulsions [24,25]. The challenge is to develop all-natural sunscreen formulations in which lignin provides multifunctionality such as emulsion stability, sun protection, antioxidant, and antimicrobial activity [26].

Mineral oils have been widely used in skincare products for decades; however, recently a significant amount of attention has focused on vegetable oils, owing to their many biological activities and sunscreen properties [27]. Until now, there are limited research studies related to the preparation of Pickering emulsions using lignin particles as stabilisers of natural oils.

In this research, lignin particles from softwood kraft lignin (SKL) were evaluated as a multifunctional ingredient for manufacturing bio-based oil-in-water (O/W) Pickering emulsions. Since the effectiveness of lignin particles in stabilising emulsions also depends on the oil phase, a preliminary screening using five natural oils and one mineral oil, varying oil/water ratio and concentration of lignin particles, was carried out to select the most suitable conditions and ingredients for the synthesis of high-quality emulsions. In addition, a physico-chemical characterisation of oils was performed, and their capacity for filtering ultraviolet rays was investigated. Based on that, three Pickering emulsion systems were formulated using rotor-stator homogenisation as a pre-emulsification step followed by ultrasonication to form fine

emulsions. The quality of elaborated bio-based Pickering emulsions in relation to the process parameters and ingredients used in the formulation was assessed. The effect of ultrasonication on the characteristics of emulsions was investigated, and the stability of the systems was evaluated over time, both visually, and microscopically. Furthermore, the *in vitro* Sun Protection Factor (SPF) of the synthesised emulsions was determined and the synergic effect between lignin particles and vegetable oils was discussed.

## 2. Experimental procedure

### 2.1. Materials

Commercial softwood kraft lignin (SKL) isolated from the Lignoboost process was supplied by a Finnish company. The technical specifications such as molecular weight distribution and purity were previously reported [28]. Lignin the lignin particles were produced in a pilot plant. The concentration of particles in the colloidal suspension was 3.50 wt%. The average size of lignin particles was 183.6 nm, and the surface charge was  $-43.3$  mV. The oils selected for the preparation of Pickering emulsions were orange oil (Sigma Aldrich), coconut oil (Sigma Aldrich), paraffin oil (Sigma Aldrich), unrefined and cold pressed organic cannabis oil (Tovarna Organika d.o.o.), unrefined and cold pressed raspberry oil (Tovarna Organika d.o.o.), and carrot seed oil (Tovarna Organika d.o.o.). The oils were protected from light until their utilization.

### 2.2. Preparation of oil-in-water Pickering emulsions

With the aim of selecting the most appropriate parameters and ingredients for the preparation of oil-in-water emulsions using lignin particles as a stabiliser, a preliminary screening was carried out based on the concentration of lignin particles in the formulation, type of oil, and oil/water ratio. The concentration of lignin particles in the water phase was fixed to 3.5, 1.75, 0.70, and 0.35 wt%. Emulsions were prepared using a stirrer-type vortex (SA8 vortex mixer, Stuart Scientific) at 2500 rpm for 2 min with oil/water phase ratios of 10/90, 20/80, 30/70, 40/60, and 50/50, and the oils mentioned in the previous section. The influence of the studied conditions was visually assessed also with a digital microscope (Keyence VHX-6000) in order to determine the key process parameters and ingredients for the synthesis of high-quality emulsions. Then, high-quality oil-in-water Pickering emulsions were formulated using three oils (orange, coconut and paraffin), oil/water phase ratios (20/80, 30/70, and 40/60), and concentration of lignin particles of 0.70, 1.75, and 3.50 wt%. The emulsification process involved two steps consisting of rotor-stator homogenisation (UT), followed by ultrasonication (US). Coarse emulsions were prepared by mixing lignin particle suspension and the selected oil with a rotor-stator homogenizer (Ika Ultra Turrax T18 digital) at 10000 rpm for 2 min at a room temperature of 21 °C. Then, the coarse emulsions were subjected to an ultrasonic processor (Hielscher ultrasonic processor UP400St) with a 14 mm diameter titanium probe immersed at a depth of 1 cm. To produce fine emulsions, the amplitude was fixed at 50 % and the sonication time was 90 s. The emulsification process was carried out in a cold-water bath at 10 °C, to avoid overheating of emulsions.

### 2.3. Analytical procedures

#### 2.3.1. Characterisation of oils

Infrared spectra measurements of oils were performed in an Alpha II spectrophotometer (Bruker Optik GmbH, Germany) equipped with a diamond crystal. The IR spectrum of investigated samples was recorded on attenuated total reflectance mode from an average of 64 scans in the 4000–400  $\text{cm}^{-1}$  range at a spectral resolution of 4  $\text{cm}^{-1}$ . The UV absorption of oils was measured by spectrophotometric analysis of dilute solutions in the range from 290 to 400 nm at 5-nm intervals using the

Mettler Toledo UV7 spectrophotometer. For this analysis, hexane was used as solvent. An initial stock solution of 1 % v/v of oil in hexane was prepared and then diluted to 0.5 %. The experimental absorbance values were collected in triplicate and used for *in vitro* Sun Protection Factor (SPF) determination using the Mansur equation [29,30].

### 2.3.2. Characterisation of Pickering emulsions

The shape, size, and distribution of droplets from both coarse and fine emulsions were analysed using the digital microscope Keyence VHX-6000 (Keyence, Osaka, Japan) in the transmission mode. In addition, fine emulsions were examined using the fluorescent imaging system EVOS M7000 (ThermoFisher Scientific) at a magnification of 100× with immersion oil and GFP light cube (excitation 470/22 nm, emission 510/42 nm). The electrical charges ( $\zeta$ -potential) on emulsion droplets were examined on the Zeta Sizer (Malvern Zetasizer Nano ZS, Malvern Panalytical, Malvern, UK). Emulsion samples were diluted 1:100 using distilled water and the measurement was carried out in triplicate in a folded capillary cell (Malvern, Zeta sizer nano series DTS1070, Malvern Panalytical, Malvern, UK), immediately after the ultrasonication step (fresh emulsion). For assessing the physical stability of synthesised emulsions, the samples were stored in the dark at room temperature (21 °C) and monitored for a period of time. The emulsion layer, defined as the emulsion height over that of the whole system, was calculated before and after the ultrasound process, and its evolution was monitored after a few days of storage.

### 2.3.3. *In vitro* Sun Protection Factor (SPF)

The best emulsion systems regarding the stability (visual phase separation, texture, and drop size) were selected for determining the sun protection parameters according to the protocol proposed by Diffey and Robson, 1989 [31], which takes into account the full UV spectrum (290–400 nm) allowing for a rapid assessment of the Sun Protection Factor (SPF) as well as the study of the product against long-wavelength ultraviolet radiation. Synthesised emulsions were applied at 2 mg/cm<sup>2</sup> to quartz slides and dried for a few minutes before UV transmittance measurements. A clean quartz slide was used as a reference. Due to the heterogeneity of the samples during the application of the emulsion on the slides, 10–20 replicas were analysed. UV transmittance was measured in a Mettler Toledo UV7 spectrophotometer from 290 to 400 nm at 5-nm intervals. The collected transmittance spectra were used to calculate *in vitro* Sun Protection Factor (SPF) (Eq. (1)).

$$SPF = \frac{\sum_{290}^{400} E_{\lambda} B_{\lambda}}{\sum_{290}^{400} \frac{E_{\lambda} B_{\lambda}}{MPF_{\lambda}}} \quad (1)$$

Where,  $E_{\lambda}$  = CIE erythral spectral effectiveness,  $B_{\lambda}$  = solar spectral irradiance, and  $MPF_{\lambda}$  = spectral transmittance of the solid substrate/spectral transmittance of the sample. In addition, to evaluate the efficiency of lignin in protecting against UVA radiation, UVA/UVB ratio and the critical wavelength ( $C_{\lambda}$ ) were calculated from the absorbance spectrum using Eqs. (2) and (3), respectively.

$$\frac{UVA}{UVB} \text{ ratio} = \frac{\sum_{320}^{400} A_{\lambda} \Delta \lambda}{\sum_{280}^{320} A_{\lambda} \Delta \lambda} \quad (2)$$

$$C_{\lambda} = \int_{280}^{C_{\lambda}} A_{\lambda} d\lambda = 0.9 \int_{280}^{400} A_{\lambda} d\lambda \quad (3)$$

## 3. Results and discussion

### 3.1. Physicochemical characteristics of ingredients

The physical and cosmetic properties of investigated oils are presented in Table 1. Paraffin oil is a transparent and odorless mineral oil widely used in the production of cosmetics or beauty products. Vegetable oils are of high interest due to their natural origin and their chemical composition that is rich in phytochemicals, and which provide beneficial properties for skincare applications.

Spectroscopic techniques such as Fourier transform infrared (FT-IR) and UV–vis spectroscopy were used to study the chemical composition and the ultraviolet filter activity of oils.

The FT-IR results of paraffin oil and natural oils are presented in Fig. 1. Liquid paraffin is a mixture of high molecular weight alkane derivatives, and the spectrum shows the characteristic peaks associated with the C–H stretching vibrations of  $-\text{CH}_3$  and  $-\text{CH}_2-$  (2925 and 2850  $\text{cm}^{-1}$ ), and peaks at 1460 and 1375  $\text{cm}^{-1}$  which correspond to the deformation of C–H in methyl and methylene groups in saturated hydrocarbon chains.

Oils from raspberry, carrot seed, and cannabis presented identical spectra in the mid-infrared region, suggesting that such oils are based on extracts in a carrier oil, which is usually olive or sunflower oil, and therefore, their spectra show typical absorption peaks of triglycerides (3010  $\text{cm}^{-1}$  and 1750  $\text{cm}^{-1}$  associated to unsaturation of fatty acids and C=O from ester bonds) [32]. Table S1 (Supporting information) summarises the band assignment corresponding to the identification of peaks in the investigated oil samples. Coconut oil has a very similar spectra to raspberry, carrot seed, and cannabis oils as it also consists of triglycerides (1750  $\text{cm}^{-1}$ , C=O). However, the band at 3010  $\text{cm}^{-1}$ , which is attributed to the stretching vibration of unsaturated fatty acids (=C–H stretch and *cis* -C=C- stretch), was not visible since coconut oil is rich in saturated fatty acids, such as lauric acid and myristic acid, and usually has very low content (< 5 %) of monounsaturated acids like oleic acid [33].

Although the chemical composition of orange oil may vary depending on the origin and extraction conditions, it is mainly composed by limonene (> 90 %) and other monoterpenes, long chain hydrocarbons and fatty acids such as linoleic, oleic and palmitic acid, alcohols and aldehydes [34–36]. Therefore, the FTIR spectrum of orange oil was characterised by limonene vibrational modes showing characteristic peaks at 885  $\text{cm}^{-1}$  (C=CH<sub>2</sub>), 1440  $\text{cm}^{-1}$  (deformation of CH<sub>2</sub>) and 1645  $\text{cm}^{-1}$  associated with the stretching of the double bonds (C=C) found in the endocyclic and exocyclic positions of the structure [37]. Additionally, in the region between 1000 and 750  $\text{cm}^{-1}$ , the peaks may

**Table 1**  
Physical and cosmetic properties of selected oils.

Name	State	Color	Specific gravity	Cosmetic properties
Paraffin oil	Liquid	Colourless	0.841	Softening and film-forming agent, solvent
Orange oil	Liquid	Yellow	0.833	Antioxidant, anti-inflammatory, antibacterial, reduce redness, astringent effect, pleasant aroma
Raspberry oil	Liquid	Yellow	0.930	Keep skin moisturised, antioxidant, anti-inflammatory, anti-aging, high levels of phytosterols
Carrot seed oil	Liquid	Orange	0.933	Powerful moisturising, soothing effect, stimulate cell regeneration, anti-aging, rich in antioxidants and vitamins
Cannabis oil	Liquid	Greenish yellow	0.910	Antioxidant, hydrating, anti-sebum, anti-aging
Coconut oil	Solid	Colourless	0.903	Hydrating, cooling, soothing effect, antioxidant

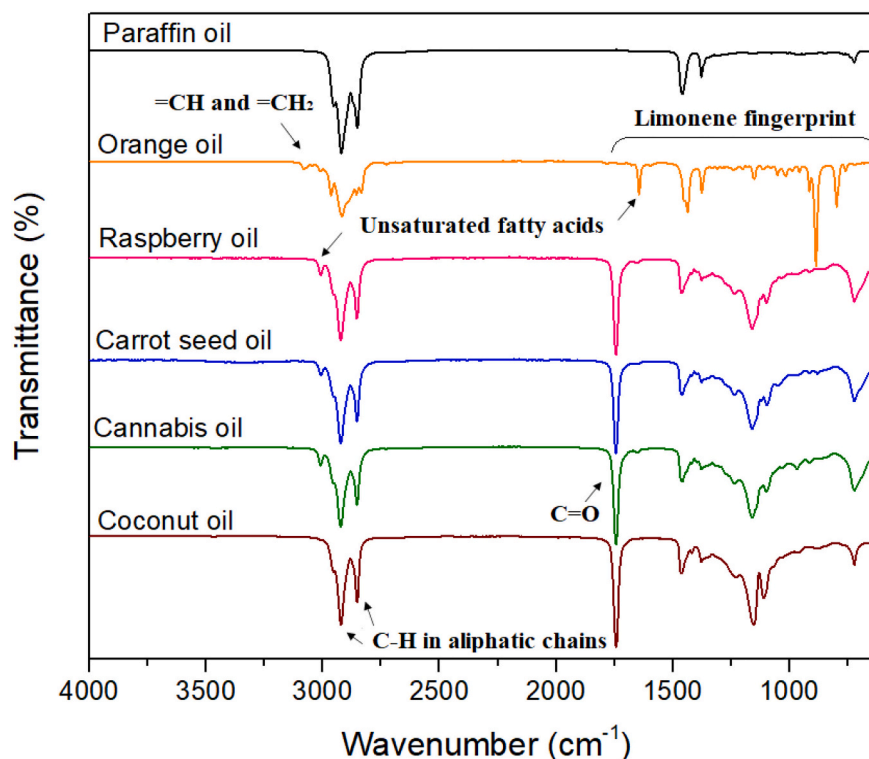


Fig. 1. FT-IR spectra of selected oils for the elaboration of Pickering emulsions.

be due to other monoterpenes which are usually present in lower concentrations such as  $\beta$ -myrcene and  $\alpha$ -pinene.

Vegetable oils such as coconut and carrot oils are currently considered as safe alternatives to commercial sunscreens, and therefore, the UV protection ability of selected oils was investigated [27,38]. The evaluation of the absorption characteristic of vegetable oils by spectrophotometric analysis of dilute solutions is a widely reported *in vitro* method in literature. However, among the described methods, there are several variations in terms of oil concentration and used solvents. In this work, oils were diluted in hexane instead of ethanolic solutions as was suggested by Mansur et al., 1986 [39] in order to avoid the turbidity caused by the low solubility of oils in ethanol. The UV-vis absorption spectra of oils in hexane and their SPF values are presented in Fig. 2 and Table 2, respectively. As expected, paraffin oil had no absorption

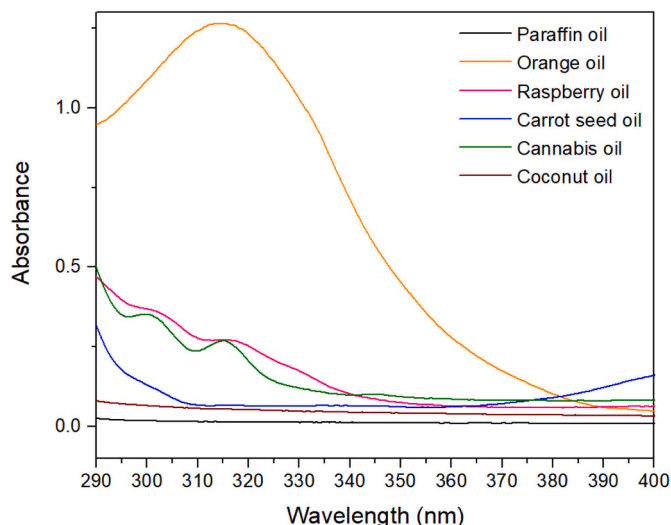


Fig. 2. UV-vis absorption spectra of oils in hexane at 0.5 % v/v.

Table 2

SPF values of oils determined by Mansur equation of diluted solutions.

Paraffin oil	Orange oil	Raspberry oil	Carrot seed oil	Cannabis oil	Coconut oil
$0.1 \pm 0.0$	$7.0 \pm 0.4$	$2.4 \pm 0.1$	$0.7 \pm 0.1$	$2.1 \pm 0.4$	$0.5 \pm 0.1$

capacity in the UV region.

The high signal to noise ratio and high sensitivity of the UV-vis region provided different signals and absorbance profiles of tested natural oils, indicating the presence of different UV-vis absorber components such as polyphenols, tocopherols, anthocyanins, or carotenoids [40]. According to the results, natural oils showed strong absorbance in the UV-C range, especially in the case of cannabis, orange, raspberry, and carrot seed oils. The absorption in this range is due to the conjugated carbon-carbon double bonds of unsaturated fatty acids [41] present in the composition of these oils as was confirmed by FT-IR spectroscopy. Coconut and paraffin oils did not present absorbance in the UV-C range.

Orange, raspberry, and cannabis oils showed UV absorption capacity in both UV-B and UV-A ranges, indicating their potential to act as a broad-spectrum sun blocker in cosmetic formulations. Orange oil exhibited higher UV shielding performance and SPF value ( $SPF = 7.0$ ) compared to raspberry ( $SPF = 2.4$ ) and cannabis oils ( $SPF = 2.1$ ). Surprisingly, coconut oil and carrot oil showed very weak effectiveness against UV radiation and low  $SPF (< 1)$  compared to the rest of the natural oils. Other studies also reported the low UV protection capacity of coconut and carrot seed oils [30,42,43]. Ácsová et al., 2021 [38] investigated the photoprotective efficacy and SPF of 14 vegetable oils by *in vitro* and *in vivo* methods and reported that most of them had no absorbance in the UV range; however, the authors observed the ability of vegetable oils to protect the skin against reddening and attributed this behavior to the components in the oil, such as tocopherols, phytosterols, carotenoids, which have anti-inflammatory, anti-erythema, antioxidant, and other biological activities.

Regarding CLPs, the wettability of spherical lignin particles was



measured to predict their behavior as a stabiliser in the formation of emulsions. CLPs were arranged on a glass surface by a dip-coating process (3 layers). The results confirmed the hydrophilic nature of CLPs used in this research, with an average contact angle value of  $9.8^\circ$  (Fig. S1). Other authors found contact angles of lignin particles ranging from 17 to  $53^\circ$  [44].

### 3.2. Screening of ingredients and emulsification process parameters

The preliminary screening of emulsification process parameters aimed at evaluating the effect of the concentration of lignin particles, the ratio between the oil and water phase, and the oil type, on the formation of emulsions, for the selection of the most suitable conditions and ingredients to synthesise high-quality emulsion systems.

The macroscopic examination evidenced that the use of a low-power mixing instrument like the vortex did not provide enough energy to the system to form stable emulsions except in the case of orange oil, which showed a totally different behavior compared to the rest of the tested oils, forming an emulsion even at a low concentration of 0.35 wt% of CLPs.

Nevertheless, the general trend with the rest of the oils evidenced that a low concentration of lignin particles in the water phase did not benefit the formation of emulsions. Systems containing 0.35 wt% and 0.70 wt% of lignin particles were not able to form stable emulsions, showing phase separation minutes after their mixture. Thus, with higher particle concentration there was better emulsification efficiency, and less phase separation was observed. Regarding the influence of oil content in the formulation, it was observed that larger amounts of oil (> 20 %) positively affected the formation of the emulsion (Fig. S2).

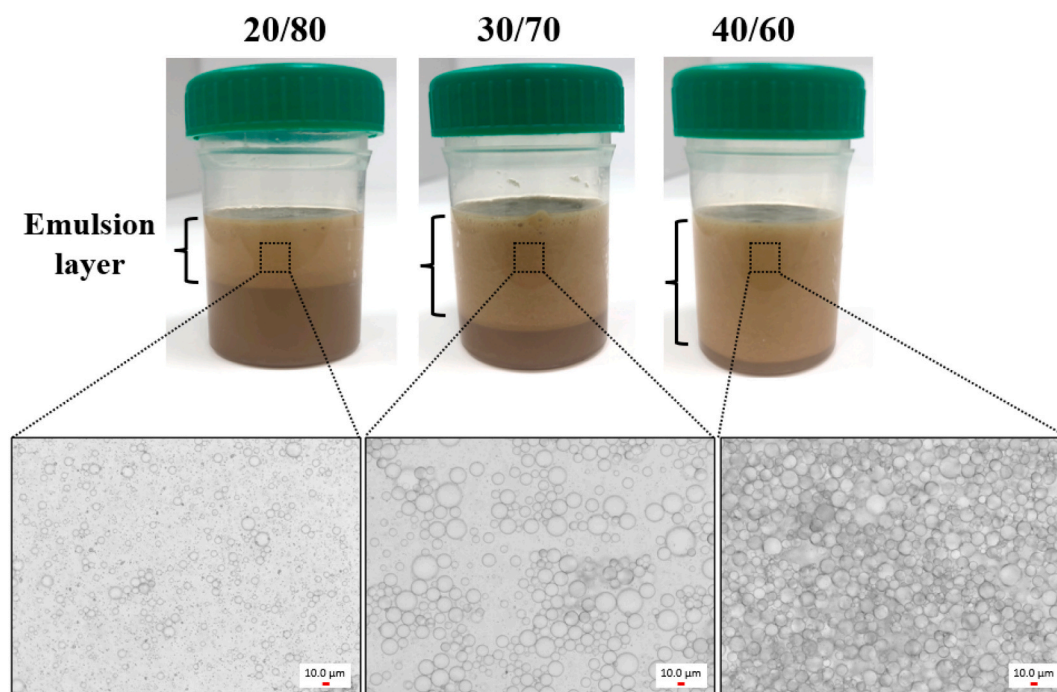
As a conclusion of this preliminary experiment and based on the characterisation of oils and their usage in the cosmetic industry, orange, coconut, and paraffin oils were selected as water immiscible phases for the next part of the research, where three Pickering emulsion systems were prepared using two-step emulsification processes (rotor-stator homogenizer followed by ultrasonication).

### 3.3. The effect of the concentration of ingredients and the process on emulsification efficiency

Three emulsion systems were prepared based on the selected oils from the preliminary screening. In this part of the study, the influence of the oil content and type, concentration of lignin particles, and the effect of ultrasonication on emulsification efficiency was investigated. In total, 27 different emulsions were obtained.

The ability of CLPs to stabilise Pickering emulsions was widely influenced by the concentration of lignin particles and oil-phase. Oil content of 20, 30, and 40 % v/v were used for the preparation of Pickering emulsion systems and the results demonstrated that higher concentrations of oil, regardless of the type of oil, favoured the formation of the emulsion, with less phase separation observed at higher oil volume fraction in the formulation. Fig. 3 shows the emulsions obtained after rotor-stator homogenisation using a water phase containing CLPs at 0.70 wt% and different paraffin oil contents.

Fig. 4 presents the emulsion layer after the first and second homogenisation steps in relation to the CLPs and oil concentration for the three studied systems. As can be observed, increasing the oil concentration in the formulation increased the emulsification layer. The same trend was observed for the rest of the oils; however, orange oil showed the best emulsification performance after the first homogenisation step even at low concentrations in the formulation. The concentration of CLPs also greatly influenced the stabilisation of oil drops and the formation of the emulsion; less phase separation was observed with the increase of CLPs concentration in the system. A phase separation after the first and second homogenisation step was only observed at 0.70 wt% concentration of lignin particles. Emulsification efficiency significantly improved with higher concentrations (1.75 and 3.50 wt%), especially after ultrasound treatment, yielding around 100 % of emulsification layer. Moreover, the use of ultrasonication as a second step of homogenisation considerably enhanced the emulsification, especially for those systems with low oil content and lignin particles.



**Fig. 3.** Pickering emulsions with different contents of paraffin oil and CLPs at 0.70 wt% after ultraturrax homogenisation (1st step) and their corresponding micrographs at  $500\times$  magnification.

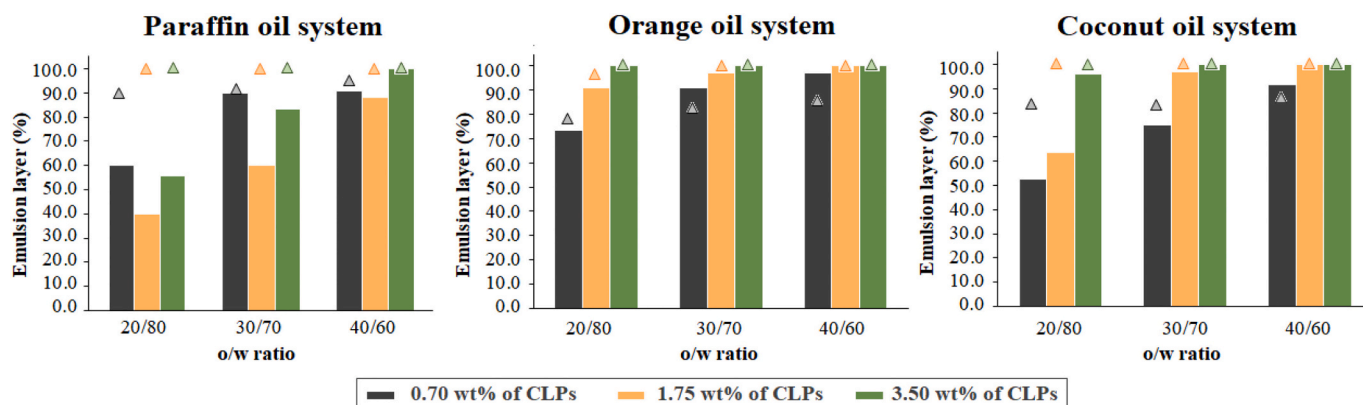


Fig. 4. Emulsion layer (%) of three emulsion systems after rotor-stator homogenisation (bars) and ultrasonication (triangles).

### 3.4. The impact of two homogenisation steps on the characteristics of emulsions

The oil content and type, the concentration of lignin particles and the homogenisation methods used significantly influenced the characteristics of the emulsions and drop size.

After the pre-emulsification step with the rotor stator, it was observed that Pickering formulations prepared with a higher oil volume fraction showed a more compact microstructure and were larger in size (see Fig. 3). Furthermore, at determined oil volume fractions, by increasing the concentration of CLPs, more homogeneous in size and smaller drops were observed in the case of orange oil (Fig. 5). However, the opposite trend was detected in the case of paraffin and coconut oil after rotor-stator process, where an increase in the CLPs concentration led to emulsions with higher heterogeneity in droplet size and the presence of non-adsorbed lignin agglomerates due to the lower oil/water interfacial area created during the emulsification process compared to the amount of Pickering particles [23] (Fig. S3).

The use of a laser diffraction instrument for the measurement of the droplet size and distribution presented several limitations in terms of reliability and replicability of the results. This could have been caused by several factors such as the presence of particles on the interface of the droplets affecting the light scattering, the presence of non-adsorbed lignin particles, and issues related to optical properties of the emulsions. Some authors assume that the droplets are completely covered by the Pickering particles, and therefore, the refractive index value of the particle stabiliser should be used for measurement. However, others support the theory that light scattering is independent of the solid particles adsorbed on the emulsion's droplets as the thickness of the stabilising layer is in the order of a few nanometers, and therefore, the refractive index value of the pure dispersed phase should be used in the measurement [45].

Therefore, in this work a qualitative optical analysis was carried out

to compare the droplet size of emulsions from the three prepared systems, assuming that the observed microscopic images only represent a small portion of the Pickering emulsions.

Pre-emulsification with a rotor stator formed heterogenous drop systems with sizes ranging from 10 to 60  $\mu\text{m}$  in the case of paraffin oil; smaller than 30  $\mu\text{m}$  for the orange oil system, and 5–50  $\mu\text{m}$  for the coconut oil system.

Regarding the impact of the second homogenisation step on the characteristics of emulsions, in general, a decrease in droplet size and an increase in droplet homogeneity was evidenced after ultrasound treatment due to the physical force that was applied to the Pickering emulsions, especially for those emulsions containing 1.75 and 3.50 wt% of CLPs (Fig. S4 and Table S2), which presented droplets below 10  $\mu\text{m}$ . Other authors who studied the stabilisation of O/W Pickering emulsions using cellulose nanocrystal as stabilisers observed that emulsions produced using only rotor stator presented polydisperse particle size distribution, while emulsions with less polydispersity and smaller droplet size were obtained using the rotor-stator followed by the ultrasound process [46].

Moreover, lignin agglomerates detected after the pre-emulsification process disappeared after ultrasonication, probably due to the increase in surface area caused by the drop size reduction, enabling the excess lignin particles to be adsorbed on the droplet surface, and thus, stabilising them.

Microscopic analysis of fine emulsions revealed that at constant oil content, increasing the concentration of lignin particles resulted not only in smaller droplets but in more homogeneous droplet size. This could be caused by the reduced particle-interface distance between particles and interface. However, as was detected after the rotor-stator homogenisation step, at constant concentration of CLPs, the increase in the dispersed phase ratio in the formulation contributed to the formation of larger droplets with a broader droplet size distribution, due to the inability of Pickering particles to stabilise a larger interfacial area

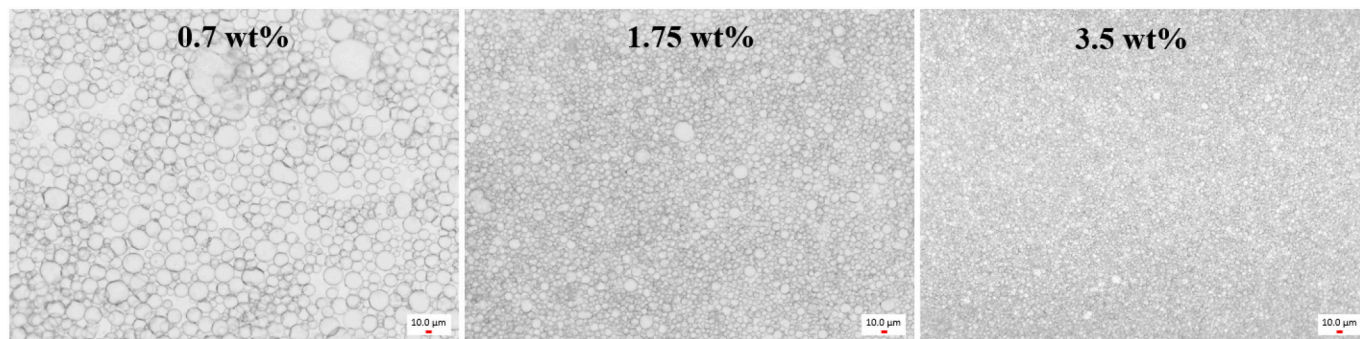


Fig. 5. Micrographs of Pickering emulsion systems with 40 % of orange oil and different concentrations of CLPs after ultraturrax homogenisation (1st step).

[23]. Other authors showed the same influence of oil content on droplet size and heterogeneity using Pickering particles of different natural origin [47]. Fig. 6 shows the images of emulsions system with orange oil obtained from the fluorescence microscope. The images revealed that lignin particles were well-adsorbed at the oil/water interface. Images from systems with paraffin and coconut oil are included in the *Supporting Information* (Fig. S5 and S6).

After ultrasonication, the systems containing low concentration of CLPs and high content of natural oils showed larger, non-spherical, and asymmetric drops (Fig. S7). This effect was due to the inability of particles to stabilise the droplets, causing a coalescence process [25]. At 1.75 wt% this effect is less evident but still noticeable in the coconut oil system, while at high concentration of CLPs (3.50 wt%) it is not noticeable. In the case of the coconut oil system at 40 % of oil and the lowest concentration of CLPs, very small droplets suspended in larger droplets were observed, evidencing the formation of a multiple emulsion. This effect was also confirmed with the fluorescent microscope images. Other authors also observed the change from simple to multiple emulsion by reducing particle concentration of graphene oxide in benzyl chloride-water Pickering emulsions [48].

Regarding the visual appearance of emulsions, at higher concentration of lignin particles in the formulations, darker color emulsions were formed, especially in the case of coconut oil systems (Table S3). In general, those produced with 1.75 and 3.50 wt% showed a brown opaque appearance and looked homogeneous, showing only one phase. Furthermore, the pH of the emulsions showed a general decrease with

the increase in CLPs concentration in the formulation, regardless of the type of oil in the emulsion system. Emulsions containing 0.70, 1.75, and 3.50 wt% of CLPs presented pH values of  $3.4 \pm 0.1$ ,  $3.1 \pm 0.1$ , and  $2.9 \pm 0.0$ , respectively.

### 3.5. Physical stability

Emulsion stability refers to the ability of an emulsion to resist changes in its physicochemical properties over time. The most common emulsion breakdown mechanisms include gravitational separation (creaming/sedimentation), droplet aggregation (flocculation), droplet coalescence, and Ostwald ripening [45].

In this work, the zeta potential of fresh fine emulsions was measured in order to predict their stability since it measures the electrical property of colloidal particles suspended in a liquid and is indicative of the attraction or repulsion of particles in a liquid suspension. The zeta potential measurements are presented in Table 3. The lowest zeta potential values were found for systems containing low concentrations of CLPs (0.70 wt%), indicating their lower ability to maintain their initial properties. The general trend showed that systems with high oil volume fraction and lignin particle concentration presented higher zeta potential value, indicating higher stability.

The Pickering emulsions were monitored over 120 days to study their physical stability. Macroscopic inspection evidenced a gravitational separation (creaming) over time for some of the synthesised Pickering emulsions, with a clarified layer at the bottom (serum layer).

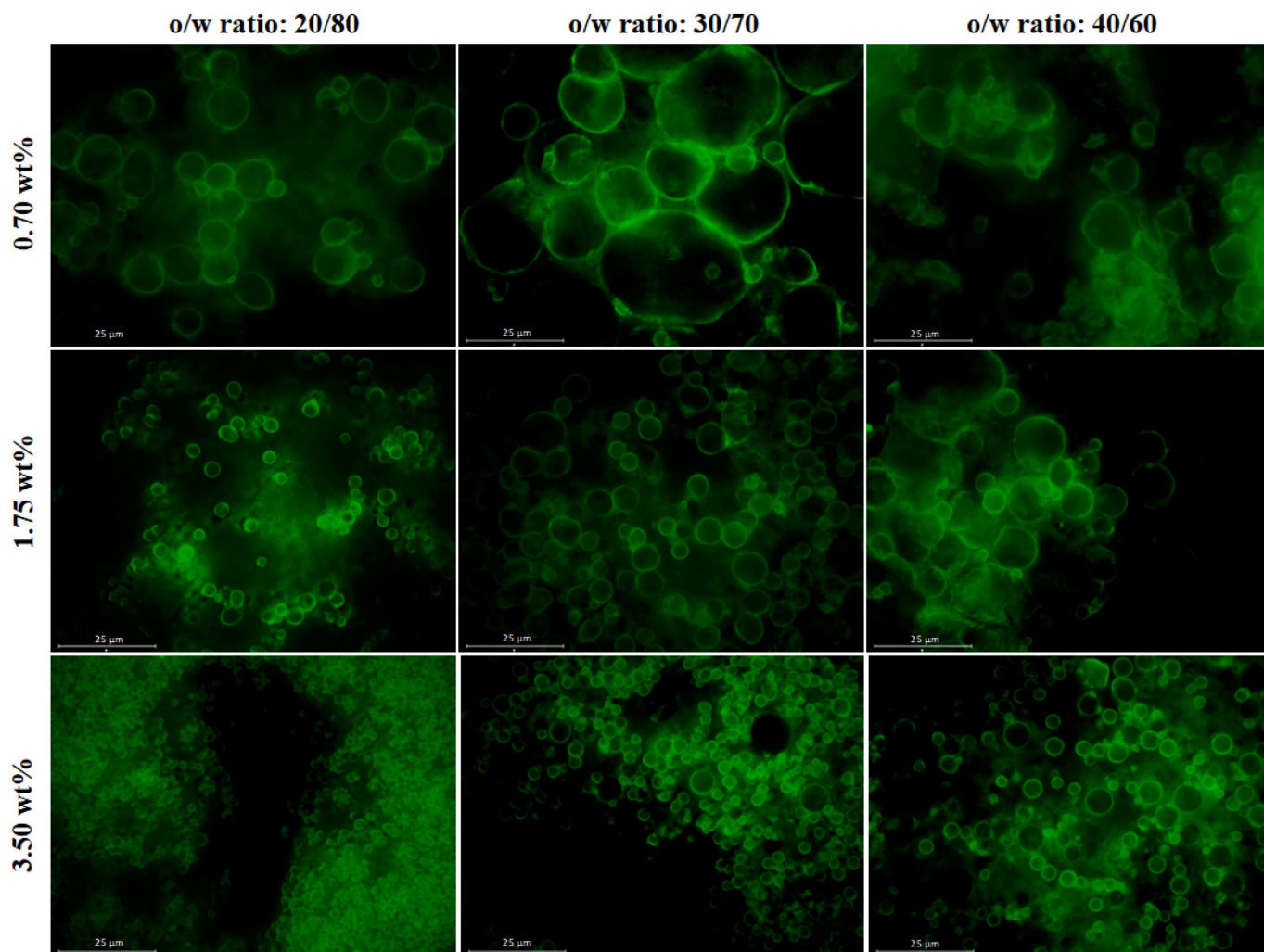


Fig. 6. Images from the digital fluorescence microscope of Pickering emulsion systems with orange oil (100×).



**Table 3**

Zeta potential of fine Pickering emulsion with paraffin, orange, and coconut oil at different o/w ratio and CLPs concentration.

Emulsion system	CLPs (wt %)	o/w ratio		
		20/80	30/70	40/60
Pickering emulsions with paraffin oil	0.70	-48.3 ± 3.6	-43.3 ± 0.6	-45.0 ± 1.1
		-54.5 ± 1.0	-49.9 ± 2.0	-57.1 ± 1.8
	1.75	-54.1 ± 1.7	-56.0 ± 0.4	-52.6 ± 0.9
		-48.9 ± 1.3	-42.2 ± 0.7	-49.4 ± 0.8
	3.50	-49.6 ± 0.5	-49.8 ± 1.3	-52.4 ± 1.9
		-42.4 ± 0.2	-47.0 ± 1.5	-50.4 ± 1.0
Pickering emulsions with orange oil	0.70	-48.4 ± 1.3	-48.5 ± 2.7	-48.3 ± 0.8
		-52.1 ± 0.5	-52.6 ± 0.7	-56.0 ± 0.7
	1.75	-53.1 ± 0.5	-52.3 ± 0.6	-55.3 ± 0.7
		-53.1 ± 0.5	-52.3 ± 0.6	-55.3 ± 0.7
	3.50	-53.1 ± 0.5	-52.3 ± 0.6	-55.3 ± 0.7
		-53.1 ± 0.5	-52.3 ± 0.6	-55.3 ± 0.7
Pickering emulsions with coconut oil	0.70	-53.1 ± 0.5	-52.3 ± 0.6	-55.3 ± 0.7
		-53.1 ± 0.5	-52.3 ± 0.6	-55.3 ± 0.7
	1.75	-53.1 ± 0.5	-52.3 ± 0.6	-55.3 ± 0.7
		-53.1 ± 0.5	-52.3 ± 0.6	-55.3 ± 0.7
	3.50	-53.1 ± 0.5	-52.3 ± 0.6	-55.3 ± 0.7
		-53.1 ± 0.5	-52.3 ± 0.6	-55.3 ± 0.7

However, it was not possible to determine the creaming index due to the optical properties of the Pickering emulsions, since the creaming layer and emulsion layer were optically opaque, making it very difficult to distinguish the boundary between the two layers. Fig. 7 represents the emulsion layer of fresh emulsions after ultrasonication, and its gradual change over time. The difference between the initial emulsion layer and the value calculated over time represented the formed serum layer.

As can be observed, the oil content and the concentration of CLPs are important factors affecting the physical stability of the Pickering emulsions. The gravitational separation and appearance of a serum layer occurred in the first 10–20 days, which remained quite stable until the end of the experimental evaluation, especially for the emulsion system containing orange oil. There was no observed phase separation in terms of the emergence of an oily layer.

The microscopic analysis (Fig. S8) did not reveal an alteration in the size of the droplets in the case of paraffin and coconut oil, confirming that no coalescence occurred. Nevertheless, in the case of orange oil, bigger droplet size has been observed specially for those systems containing low concentration of CLPs. The least stable Pickering emulsions were those prepared with low concentration of CLPs and 20–30 % of oil. Systems containing 3.50 wt% of lignin particles and 40 % of oil presented the best performance in terms of stability (no serum phase was formed during the testing period), and therefore, were selected for the SPF test.

### 3.6. Sun Protection Factor (SPF) of high-quality emulsions

The chemical structure of lignin, based on phenylpropane units linked through ether and carbon-carbon bonds, contains a wide variety of functional groups (chromophores and auxochromes) capable of absorbing UV radiation. Moreover, in contrast to the majority of chemical UV filters, lignin acts as broad-spectrum sun blocker, providing protection against both UVA and UVB radiation [16]. However, lignin on its own showed insufficient capacity to improve the UV protection performance of cosmetic formulations [16,49]. The most recent works have reported several strategies to improve lignin performance in skincare application by its functionalisation [50–52].

Recently, an interesting synergic effect between lignin and organic UV filters has been evidenced, which allows for the reduction in the amount of chemical UV filters in formulations by the incorporation of lignin [53,54]. Lee et al., 2020 [55] incorporated 5 % of lignin nanoparticles in a moisturising cream, chemical sunscreen with SPF of 15 containing aromatic compounds as active ingredients, and physical sunscreen with SPF of 15 containing zinc oxide. The SPF of the moisturising cream slightly increased to 4.3 but no significant increase was observed in the case of sunscreen containing mineral filter. However, the UV protection factor value of organic sunscreen approximately doubled due to their synergistic effect with aromatic active ingredients. Other authors proved that encapsulating the chemical sunscreen into lignin potentially strengthens the synergistic effect due to closer interaction of the chemicals [56].

No research studies have been found that examined the existence of a synergic effect between lignin particles and vegetable oils in a Pickering emulsion system. Therefore, in this study the UV protection capacity of the three selected, high-quality emulsions was evaluated according to the *in vitro* SPF method based on UV spectrophotometric method [31]. Fig. 8 exhibits the average UV transmittance of Pickering emulsions containing 3.50 wt% of CLPs and 40 % oil paraffin, orange, and coconut oils, while SPF values, UVA/UVB ratio, and critical wavelength were determined and presented in Table 4.

An improvement in the SPF values was observed in the case of Pickering emulsions prepared with natural oils with respect to the UV protection ability determined for pure oils, while no effect was observed in the case of paraffin oil. No UV absorbance was detected for paraffin and coconut oil; however, the Pickering emulsion containing coconut oil showed a sunscreen property. As was previously described, paraffin oil is a mixture of higher molecular weight alkane derivatives, while coconut oil aside from the lipophilic fraction contains some aromatic compounds, mainly phenolic acids like vanillic, gallic, syringic, and p-coumaric acids, and low content of tocopherols and  $\beta$ -sitosterol [57]. The Pickering emulsion made with orange oil showed the best UV shielding performance, due to its good initial UV absorbance compared to the rest of the oils. This oil is mainly based on terpenes (limonene, myrcene, and

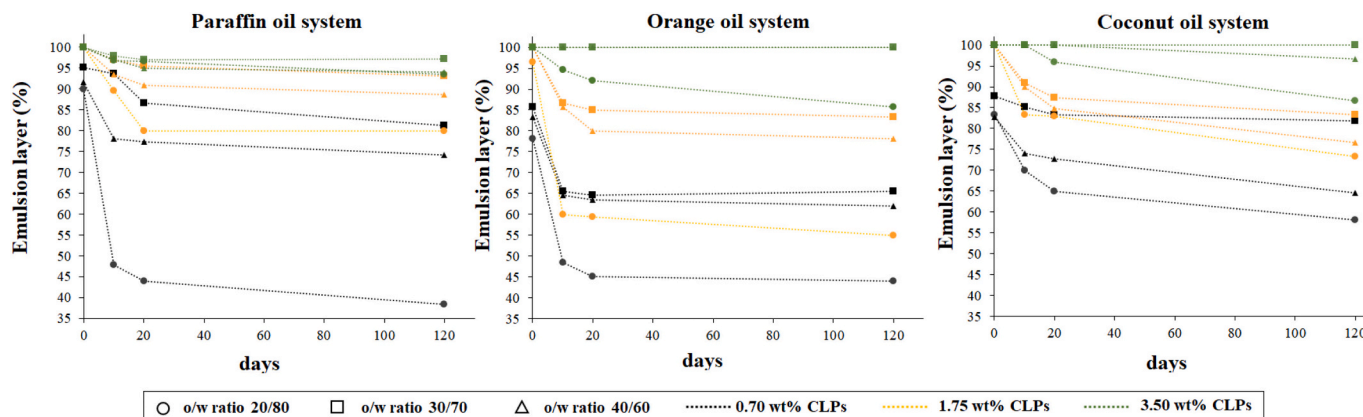
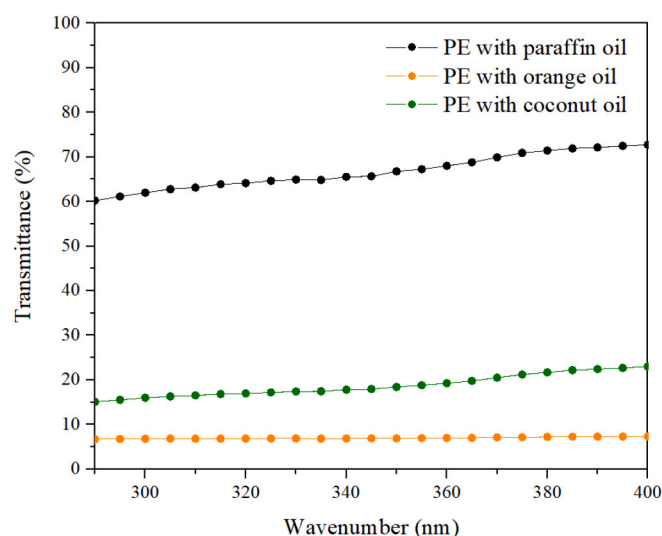


Fig. 7. Evolution of the Pickering emulsions over 120 days.





**Fig. 8.** UV transmittance of Pickering emulsions prepared with 3.50 wt% of CLPs and 40 % of oil.

**Table 4**

SPF, UVA/UVB ratio, and critical wavelength values ( $C_d$ ) of Pickering emulsions prepared with 3.50 wt% of CLPs and 40 % of oil.

Emulsion system	SPF	UVA/UVB	$C_d$
Paraffin oil	$1.5 \pm 0.3$	0.74	380.6
Orange oil	$13.7 \pm 2.8$	0.90	389.6
Coconut oil	$5.6 \pm 1.7$	0.81	390.8

$\alpha$ -pinene), but also usually contain low amounts of oxygenated compounds, carbonyl compounds, alcohols, and esters [58]. Moreover, the broad-spectrum protection capacity of prepared emulsion systems was demonstrated by the critical wavelength value, defined as the wavelength at which the integral of the spectral absorbance curve reached 90 % of the integral from 290 to 400 nm, and the UVA/UVB ratio [59].

Based on these results, the incorporation of phenolic compounds with great UV absorption ability into bio-based oil-in-water Pickering formulations through encapsulation could contribute to the improvement of the SPF value of emulsions.

#### 4. Conclusions

Lignin particles from softwood kraft lignin were evaluated as a multifunctional ingredient for manufacturing high-quality oil-in-water Pickering emulsions. The research was carried out in two steps in order to compressively evaluate the stability of lignin particles in the developed emulsions. First, a preliminary screening using five natural oils and one mineral oil, varying oil/water ratio, and different concentration of lignin particles, was carried out to select the most suitable conditions and ingredients for the synthesis of high-quality emulsions. Based on the screening, physico-chemical characteristics, cosmetic properties, and industrial interest of oils, orange, coconut, and paraffin oils were selected as water immiscible phases for the next part of the research, with an oil phase content between 20 and 40 % and concentration of CLPs at 0.70, 1.75, and 3.50 wt%. Thus, three Pickering emulsion systems were formulated using two-step homogenisation processes: rotor stator homogenisation followed by ultrasonication. The ability of CLPs to stabilise Pickering emulsions was widely influenced by the concentration of lignin particles, oil-phase, and used emulsification process. Higher concentration of oil and CLPs in the formulation was positively correlated with increased emulsification. In addition, the use of ultrasonication as a second homogenisation step considerably enhanced the

emulsification, especially for those systems containing low oil and content of lignin particles. The microscopic characterisation revealed that emulsions produced using only rotor stator presented polydisperse particle size distribution, while application of ultrasonication as a second homogenisation step generated emulsions with less polydispersity and smaller droplet size. The micrographs demonstrated that at constant oil content, increasing the concentration of lignin particles resulted not only in smaller droplets but in a more homogeneous droplet size. However, at a constant concentration of CLPs, an increase of the dispersed phase ratio into the formulation contributed to the formation of larger droplets with a broader droplet size distribution. Moreover, systems with higher oil volume fraction and lignin particle concentration presented higher physical stability, without phase separation after 120 days of monitoring. Regarding the sun protection capacity of emulsions, an improvement in the SPF values was observed for Pickering emulsions prepared with natural oils with respect to the UV protection ability determined for the pure oils, indicating an interesting synergic effect between chemical components present in tested vegetable oils and lignin particles. Therefore, this research proves the multifunctionality of lignin particles in a formulation, where they not only act as an emulsifier but also behave as a UV radiation filter booster when combined with natural oils rich in aromatic compounds. However, the size and origin of lignin particles could considerably influence the emulsification performance as well as the SPF results, and it should be investigated in future works.

#### CRedit authorship contribution statement

**Oihana Gordobil:** Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Resources, Project administration, Supervision, Funding acquisition, Writing – original draft, Writing – review & editing. **Nicole Blažević:** Methodology, Investigation, Validation, Formal analysis. **Marjana Simonič:** Writing – review & editing. **Anna Sandak:** Project administration, Writing – review & editing.

#### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijbiomac.2023.123561>.

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