



Article Chemical Stabilization behind Cardamom Pickering Emulsion Using Nanocellulose

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Abstract: Cardamom essential oil (EO) is a rare oil of high scientific and economic interest due to its biofunctionality. This work aims to stabilize the EO by Pickering emulsions with nanocellulose, in the form of nanocrystals (CNC) or nanofibers (CNF), and to investigate the stability and chemical and physical interactions involved in the process. The emulsions were characterized by droplet size, morphology, stability, surface charges, Fourier transform infrared spectroscopy, FT-Raman, nuclear magnetic resonance, and scanning electron microscopy. Stable emulsions were prepared with cellulose morphologies and CNCs resulted in a 34% creaming index, while CNFs do not show instability. Emulsions indicate a possible interaction between nanocellulose, α -terpinyl acetate, and 1,8-cineole active essential oil compounds, where α -terpinyl acetate would be inside the drop and 1,8-cineole is more available to interact with cellulose. The interaction intensity depended on the morphology, which might be due to the nanocellulose's self-assembly around oil droplets and influence on oil availability and future application. This work provides a systematic picture of cardamomum derived essential oil Pickering emulsion containing nanocellulose stabilizers' formation and stability, which can further be extended to other value-added oils and can be an alternative for the delivery of cardamom essential oil for biomedical, food, cosmetics, and other industries.

Keywords: cardamom essential oil; cellulose nanocrystals; cellulose nanofibers; oil-in-water emulsion; stability

1. Introduction

Cardamomum (*Elettaria cardamomum*) is a perennial shrub with thick, fleshy lateral roots that can grow to eight feet in height. It is one of the most used spices globally, and as a cooking seasoning, the darker seeds are removed from the pod and ground into powder. Cardamomum is grown mainly in southern India, Sri Lanka, Tanzania, and Guatemala [1,2]. Seeds and essential oil derived from cardamomum are used as a flavoring component in various foods, including beverages, frozen desserts, sweets, baked goods, sauces, and meat products. This widespread use directly in food reinforces that it is safe for health [3]. In addition to culinary uses, cardamomum has been used in medicine for years, such as for asthma control, cardiac and digestive disorders, nausea, and diarrhea. Besides, its essential oil (EO) and bioactive metabolites are biofunctional and can be used in the pharmaceutical, food, and nutraceutical industries [4]. The cardamomum EO's composition has mostly 1,8-cineole (20–60%) and α -terpinyl acetate (20–55%) compounds [5,6]. Other monoterpenes can be found in minor quantities, such as α -pinene, linalool, and linalyl acetate. Despite its numerous benefits, this EO is sparsely used worldwide because it is a high-cost oil with low stability.



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Cardamomum EO's instability results in high volatility, hydrophobicity, intense aroma, photosensitivity, high oxidation susceptibility, and solubility. Among the main approaches used in the literature to improve its stability, encapsulation (micro and nano) [7,8], nano-emulsification, and Pickering emulsions can be highlighted [9,10]. Pickering emulsions, named after Pickering's work [11], were first observed by Ramsden [12]. Ramsden, in his work, used highly viscous material to cover the surface of two-phase solutions, examining the solid material's ability to reduce the system surface tension and energy. Pickering emulsions use solid particles, natural or synthetic, to stabilize the oil/water interface [13]. Like the surfactants, these solids decrease the free energy that needs to be overcome for system stability. According to Ortiz et al., the solid phase's chemical composition, morphology, and concentration will directly impact the emulsion's stability [14]. Among the most common natural nanoparticles that could act as solid particles for stability are cellulose, chitin, and chitosan, which, in addition to being suitable stabilizers, are also sustainable, biocompatible, and biodegradable [15,16].

Nanocelluloses have three main types: nanocrystals (CNC), nanofibers (CNF), and nanoparticles (NPs). CNC has abundantly available hydroxyl groups, a low aspect ratio, high crystallinity, and stiffness [17]. CNFs are characterized by a high aspect ratio (micrometric lengths and nanometric diameters), flexibility, and high mechanical strength [18]. NPs are cellulosic materials with different morphologies, such as ribbon-like or short needle particles that contain amorphous and crystalline regions in their composition [19]. The mechanical resistance, biocompatibility, nontoxicity, and potential for surface-chemistry modification of all nanocellulose types result in simultaneous steric and electrostatic emulsions stabilization, resulting in high stability for months, with resistance to temperature and shear [20].

Ma et al. (2022) prepared CNC emulsions for 3D printing, containing 0.5 wt% of solid particles, and obtained a system with excellent storage stability [21]. The authors attributed the emulsion's stability to the emulsion's viscoelasticity, which allowed for their application to 3D printing materials for nutrition delivery systems, food manufacturing, and biomedical tissue engineering. Li et al. (2022) developed Pickering emulsions stabilized with CNC or CNF to evaluate in vitro gastrointestinal digestibility, aiming at a food-grade emulsion, and verified that cellulose nanofibers decrease the degree of lipid digestion compared to CNCs due to the difference in the nanocellulose self-assembly at the interface. Since CNFs form an interconnected network that encapsulates the oil, its release is compromised; however, it can be helpful in the development of slow-release bioactive systems [22].

The interfacial adsorption or attachment to the oil phase is a significant factor influencing the emulsion's stability. Besides the morphological influence, Bertsch and collaborators reported that the essential oil's chemical structure, polarity, and processing parameters influence how nanoparticles will be positioned around the droplets and the stability mechanism [23]. However, a few studies have investigated this approach in-depth concerning adsorption and emulsification. Chen et al. (2016) investigated the relationship between cellulose nanocrystals' deformation and anisotropy concerning bending characteristics, which could alter the accommodation of CNCs around oil droplets' curvature. According to the authors, the crystalline planes of cellulose favor their adsorption at the oil/water interface and, consequently, promote the emulsions' stability [24]. Fanch et al. (2016) described the interface of CNC–Pickering emulsions and reported that cellulose crystalline plans directly interact with oil, and this interaction is limited to the interfacial contact [25].

Furthermore, the literature presents several works on developing emulsions with different essential oils by varying homogenization methodology, solid particle, essential oil, and processing parameters. However, cardamom essential oil is rarely studied due to its main applications in the food sector. Nevertheless, this oil has tremendous potential for application in several areas if adequately stabilized. Unfortunately, few works carry out an in-depth study of the chemical interactions occurring in an emulsion, such as new chemical bonds between the components, that can influence the system's stability. This work aims to conduct an in-depth investigation of cardamom essential oil's emulsification

via a Pickering emulsion approach using nanocellulose, in two different morphologies, as a solid phase. The emulsions were tested by droplet size, morphology, stability, zeta potential, Fourier transform infrared spectroscopy, FT-Raman, nuclear magnetic resonance, and scanning electron microscopy.

2. Materials and Methods

2.1. Materials

According to our previous works, cellulose nanocrystals (CNC) were prepared and characterized [26,27]. CNCs had an average length of $2.0 \pm 0.8 \mu$ m and mean diameter of 131.1 \pm 29.6 nm (L/D = 15), and ζ potential of $-33.0 \pm 1.0 \text{ mV}$ [28]. Cellulose nanofibrils (CNF) were kindly provided by Suzano Papel e Celulose (Suzano, São Paulo, Brazil). CNFs presented a diameter of 66.3 \pm 20.5 nm and length of 4.0 \pm 1.0 μ m (L/D = 60), and ζ potential of $-33.2 \pm 3.2 \text{ mV}$ [29]. The CNC and CNF, at a concentration of 1.5 wt%, were stored in a refrigerator until their use. Ferquima Indústria e Comércio Ltd.a. (Vargem Grande Paulista, São Paulo, Brazil) provided the Cardamomum essential oil (*Elettaria cardamomum*, CAS Number 8000-66-6).

2.2. Emulsion Preparation

Nanocellulose (CNF or CNC) was mixed with water to adequately dilute the suspensions to 0.5 or 1 wt% and ultrasonicated (40 W, 23 kHz) for 2 min in an ultrasonic bath to prepare a dispersed aqueous solution (0.5 or 1 wt%). The nanocellulose concentration was selected based on an extensive literature search and several experimental tests previously conducted in this area [29,30]. Then, cardamom essential oil was added for the Pickering emulsion's preparation. The mixtures were prepared, using an Ultra-Turrax Blender, IKA T25 model (IKA Werke, Staufen, Germany), with 30% oil and 70% water for 7 min. The nomenclature of the prepared emulsions is presented in Table 1 along with their preparing conditions.

Table 1. Adopted nomenclatures of the prepared samples, considering their preparation methodologies and conditions: nanocellulose concentration, oil:water proportion, speed, and time.

Nomenclature	Solid Phase Concentration	Oil:Water Proportion	Speed	Time
30Car-CNC	0.5% CNC	30/70	10,000 rpm	7 min
20Car–CNC	0.5% CNC	20/80	10,000 rpm	7 min
30Car-CNF	1% CNF	30/70	12,000 rpm	7 min
20Car–CNF	1% CNF	20/80	12,000 rpm	7 min

2.3. Characterization

2.3.1. Optical Microscopy

The droplet microstructures were analyzed under a Leica DM KM optical microscope (Leica Microsystems GmbH, Wetzlar, Germany). An emulsion droplet was added to a glass slide, and representative images were obtained. The droplet diameters were measured using the ImageJ software as an average of 150 droplets.

The measurements were reported as mean \pm standard deviation. One-way analysis of variance (ANOVA) was applied, and the Tukey test was used to evaluate the significant difference between samples, using PAST software (version 4.03) and a confidence level of 95%.

2.3.2. Stability under Storage and Towards Centrifugation

Fresh samples were added to glass tubes and stored at room temperature, and the emulsion stability was accompanied for a month. The creaming index (CI) was calculated following Equation (1), where H_{cream} is the cream layer height and H_{total} is the total height.

$$CI = \frac{H_{cream}}{H_{total}} * 100$$
(1)

Centrifugation stability was evaluated after 2 h of preparation, when the fresh emulsions were centrifuged (Thermo Scientific 75,002,426, Thermo Fisher Scientific, Waltham, MA, USA) for 1 min at 12,000 rpm, following the Gestranius et al. method [19]. The emulsions were evaluated visually, and the stability was calculated according to Equation (2), where EE is emulsification efficiency, HE is the height of the emulsified phase, and HT is the total height of the sample.

$$EE = \frac{H_E}{H_T} * 100 \tag{2}$$

2.3.3. Zeta Potential

The emulsion's Zeta (ζ) potential was determined using electrophoretic mobility measurements and conversion of values using the Helmholtz–Smoluchowski equation. Zetasizer Nano ZS equipment (Malvern Instruments, Malvern, United Kingdom) was used. The emulsions were diluted at a concentration of 0.05% (v/v).

2.3.4. Fourier Transform Infrared Spectroscopy

FTIR with an attenuated total reflectance diamond accessory was performed on Frontier 94,942 equipment (PerkinElmer Inc., Waltham, MA, USA). The spectra were collected with 4 cm⁻¹ spectral resolution, 32 scans, from 4000 to 400 cm⁻¹.

2.3.5. FT-Raman

Data were obtained in an FT-Raman—MultiRaman (Bruker Optics Inc., Billerica, MA, USA) equipped with a wavelength of 1064 nm and a laser of 500 mW. Data acquisition was performed in 500–4000 cm⁻¹, 128 scans, and 4 cm⁻¹ spectral resolution.

2.3.6. Nuclear Magnetic Resonance

1D (¹H &¹³C nuclei) and 2D (HSQC—Heteronuclear Single Quantum Coherence and HMBC—Heteronuclear Multiple Bond Correlation) experiments were done to obtain nuclear magnetic resonance spectra in the liquid phase, using a 400 MHz spectrometer AVANCE III HD (Bruker Optics GmbH & Co. KG, Ettlingen, Germany) with a wideband 5 mm probe. Both EO and emulsions were diluted at 10% (v/v) with D₂O and CDCl₃ for acquisition in both deuterated solvents. The spectra were processed and analyzed using the software TopSpinTM 3.6.3 (Academia License, Bruker Optics GmbH & Co. KG, Ettlingen, Germany).

2.3.7. Scanning Electron Microscopy

The emulsions were dried at room temperature until the oil and water evaporated. After that, dried samples were coated with a 15 nm thick gold layer using Sputtering Leica EM ACE 200 (Leica Microsystems, Wetzlar, Germany). Micrographs were performed using a microscope FEI Quanta 250 (Thermo Fisher Scientific, Hillsboro, OR, USA), using an accelerating voltage of 10 kV and a spot size of 4 nm.

3. Results and Discussions

3.1. Droplet Size and Stability

Figure 1a shows the optical microscopy images of all Pickering emulsions. The samples showed characteristic emulsion morphology with spherical droplets. The mean diameters of 30Car–CNC, 20Car–CNC, 30Car–CNF, and 20Car–CNF were 58.0 \pm 32.4,

 68.1 ± 32.4 , 131.8 ± 75.4 , and $196.1 \pm 82.5 \mu$ m, respectively. The CNC emulsions were statistically similar, as well as the CNF emulsions. However, the CNC and CNF emulsions' diameters were statistically different. The oil/water proportion did not influence the droplet size, indicating that the adopted emulsification conditions were sufficient to form stable microdroplets for both oil/water ratios.



Figure 1. (a) Representative optical microscopy images, (b) digital photographs obtained from stability on days 0 and 30, and (c) stability after centrifugation of the prepared cardamom emulsions stabilized with CNC or CNF.

The small droplet sizes verified to CNC emulsions can be attributed to the emulsification mechanism, where the CNCs were probably adsorbed at the oil/water interface, resulting in a droplet surface coverage [20]. As an intrinsic characteristic of CNCs, there is the electrostatic repulsion due to the OH- groups, which ensures that the properly coated droplets do not come too close and generate instability phenomena, such as coalescence, guaranteeing small droplet sizes [21]. The CNF emulsions, on the other hand, were stabilized by droplets' immobilization inside the pores of the three-dimensional network formed by the CNFs, which causes an approximation of the drops that occupy the same space and may result in their coalescence [19,22]. Drops in different porous spaces cannot approach due to the steric barrier resulting from the cellulosic fibers. Larger droplets do not represent an unstable state but rather an emulsion with larger-scale droplets and, consequently, smaller surface area.

Figure 1b shows the digital photographs obtained for the Pickering emulsions on days 0 and 30. After thirty storage days, the CNC emulsions showed a visible phase separation, with cream and transparent layers. The creaming index of 30Car–CNC and 20Car–CNC was 34.2% for both samples, confirming that the oil concentration did not influence emulsion stability. It is expected that the interfacial film formed by the CNCs around the oil drops was ruptured due to the tendency of the system to return to the initial

thermodynamic equilibrium, which is the condition of phase separation [23]. The CNF samples showed a distinct appearance, without the formation of a creaming layer and with the aspect of a gelled structure, confirming the theory about the larger droplet sizes.

Figure 1c shows the stability towards shear of all studied emulsions. This property is related to interactions between the phases present in the system (essential oil, water, and nanoparticles) and the presence of agglomerates, which can be segregated into separate units, forming larger clusters due to droplets' coalescence [22,24]. The results showed that CNC emulsions were stable against shear, probably due to the chemical interactions between CNCs and the oil/water interface, presented in the following sections. On the other hand, the CNF-stabilized emulsions showed a slight tendency of phase separation, with stability loss of ~10%, generating an oily layer in the upper phase of falcon tubes. In this case, oil drops that were not connected and stabilized tended to separate and, due to density difference, rise to the top of the total volume. These results indicate that, even with the predominantly amphiphilic character of cellulose, emulsions present high stability (~90%) and a tendency to adsorption at the oil/water interface.

3.2. Rheology

The fluidity of a Pickering emulsion is critical for preparation and storage and for the final product. Figure 2 shows the interfacial viscosity curves as a function of shear rate of cardamom essential oil and its emulsions. The oil and the CNC-stabilized emulsions showed a Newtonian behavior, while the CNF emulsions presented a shear-thinning behavior. The Newtonian behavior of 30Car–CNC and 20Car–CNC can be attributed to favorable droplet homogeneity and emulsion stability, without the formation of microphases after the shear increasing [25].



Figure 2. Curves of viscosity versus shear rate of cardamom essential oil and its Pickering emulsions, and the obtained parameters for the Herschel–Bulkley rheological model.

PECar–CNF emulsions showed a non-Newtonian shearing behavior associated with the breaking of the tangled network of nanofibers and the orientation of the fibrils along the flow lines under the shear force [26,27]. At low shear rates, oil droplets are held together due to insufficient hydrodynamic forces to break system bonds, resulting in high viscosity. As the shear rate increases, hydrodynamic forces break the bonds and deform the CNF network and oil droplets, resulting in lower viscosity [28,29]. This behavior is directly related to the chemical bonds present between the emulsion components.

For the CNF emulsions, the Herschel–Bulkley rheological model was applied, and the parameters are presented in Figure 2, which indicate that, at rest, the materials have the formation of structures similar to a solid, possibly indicating that there are complex interactions between the components of the emulsions at the interfaces between the droplets of oil and water [30,31]. Both samples showed n values between 0 and 1, confirming the pseudoplastic behavior [32], the most common non-ideal behavior exhibited for commercial emulsions, since low n values facilitate oil dispersion and improve its bioactivity [22,27]. In addition, K values higher than n values indicate that the emulsion is not fluid under the action of external forces; that is, even under shear, the gelled aspect is maintained and, consequently, the stability [33].

3.3. Zeta Potential

The ζ potential values are associated with the ionization of surface groups, small molecules or ion adsorption, continuous charges associated with crystalline structures, or this combination of mechanisms [34]. Emulsions with high absolute ζ potential (above 30 mV) are electrically stabilized, while emulsions with low values tend to coagulate or flocculate, eventually leading to phase separation. However, the oily phase chemical structure and its functional groups can also alter these values. In addition, viscous emulsions, i.e., gel-like materials, commonly exhibit minor zeta potential associated with the stabilization type, i.e., a physical impediment [35].

Samples 30Car–CNC and 20Car–CNC showed ζ potential values of -45.6 ± 3.6 and -49.9 ± 3.9 mV, respectively, and were considered high electrostatic stable samples. Considering the standard deviation, both samples showed similar electrostatic behavior associated with the CNC's efficiency of droplet coating and their adsorption at the oil/water interface. These values are higher than the ζ potential of CNCs (–33 mV), and therefore, it is expected that there was a contribution from the essential oil's chemical structure. The α -terpinyl active compound presents a reactive OH group, and the α -terpinyl acetate has an ester as a terminal group and a benzene ring in its structure, which may be responsible for the increase in the negative charges.

The CNF emulsions presented significantly lower absolute values than CNC emulsions, and samples 30Car–CNF and 20Car–CNF showed ζ potential values of -18.3 ± 1.4 and -11.1 ± 0.9 , respectively, possibly due to the difference in the emulsion's stabilization mechanism. Cellulose nanofibers have negative electrostatic charges associated with free hydroxyls, but in less abundance. The emulsion's stability is attributed to forming a thin layer of CNFs around the oil droplets, which self-assemble into a three-dimensional gel-like structure due to nanofibers' self-organization characteristics. For the preparation methodology adopted, this mechanism proved to be the most efficient for the induction of irreversible adsorption, which prevents the drops' coalescence and results in emulsions with a stability of 100% over months of storage [36].

3.4. Fourier Transform Infrared Spectroscopy

Figure 3a shows the complete FTIR spectra of cardamomum essential oil and its CNC emulsions. The characteristic peaks of cardamomum essential oil are present in Table 2.



Figure 3. FTIR spectra of cardamomum essential oil and its CNC-stabilized emulsions (**a**) full-spectrum and zoom in the region (**b**) 1800–1425 cm⁻¹, and of CNF-stabilized emulsions (**c**) full-spectrum and zoom in the region (**d**) 1800–1425 cm⁻¹.

Table 2. Cardamom FTIR characteristic	peaks and	their respectiv	e assignments
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Peak (cm ⁻¹⁾	Assignment	Reference
2973	CH elongation	[37,38]
2922, 2883	carbonyl group elongation	[37,38]
1729	C=O carbonyl ester elongation vibrations	[37,38]
1464, 1446	CH ₂ bond	[37,38]
1366, 1306	CH ₂ bond	[37,38]
1252	carboxylic acid/acetate elongation	[37,38]
1212, 1054, 1017	СО	[39,40]
1159	CC vibrations of the molecule skeleton	[39,40]
1078	OH group vibrations	[39,40]
985	CH bending vibration absorption	[39,40]
940	CH vibrations aromatic ring	[39,40]
918	CH bond out of plane	[39,40]

The bands in the region between 888 and 770 cm⁻¹ are associated with the presence of C=C and =CH of aromatic rings and CH out-of-plane vibrations, consistent with the chemical structure of the active compounds α -terpinyl and α -acetate terpinyl, the main active compounds present in the cardamonum essential oil [41,42].

After emulsification, there was a significant change in the FTIR spectra, possibly due to the significant difference in chemical structures between CNCs and cardamomum EO. Figure 3b shows a zoom of the spectra in the 1800–1425 cm⁻¹ region. The peak 1729 cm⁻¹ almost disappeared for 20Car–CNC, indicating a conformational change in the α -terpinyl acetate chemical structure, possibly due to CNCs and water, since the band appears conjugated with the 1640 cm⁻¹. The 1470–1460 cm⁻¹ band (CH₂ bonds) decreased in intensity after the CNCs' addition, indicating a possible chemical interaction between the emulsion components.

The CNF-stabilized full spectra are shown in Figure 3c, and a zoom of the 1800–1500 cm⁻¹ region is shown in Figure 3d. The same trend is shown for peaks 1729 and 1640 cm⁻¹ as for the CNC emulsions and a new shoulder at 1374 cm⁻¹ (Figure 4a), indicating a possible chemical interaction between the emulsion components.



Figure 4. FTIR spectra of cardamomum essential oil and its CNF-stabilized emulsions: zoom in the regions (a) $1400-1275 \text{ cm}^{-1}$, (b) $1120-1000 \text{ cm}^{-1}$, and (c) $950-750 \text{ cm}^{-1}$.

The EO peaks centered at 1366 cm⁻¹ (CH₂ bonds) and 1113 cm⁻¹ (CO elongation vibrations—Figure 4b) showed a displacement of ~2 cm⁻¹ for smaller wavenumbers, indicating that the CNFs' addition alters the EO molecules' conformational organization,

possibly due to reorganization caused by steric impediment and electrostatic charges resulting from the cellulosic structure.

The peaks at 941 cm⁻¹ (aromatic ring vibrations) and 798 cm⁻¹ (C=C and =CH of aromatic rings and CH out-of-plane vibrations) (Figure 4c) showed a displacement of 2 and 4 cm⁻¹, respectively, for higher wavenumbers. These shifts can result from the bathochromic effect, i.e., the absorption band undergoes a shift due to a substitution in the molecule by another radical, or even due to an alteration in the electronic clouds surrounding the nuclei altered in the presence of other molecules through electrostatic interactions. Thus, there was no chemical bond between the components, but the spectra indicate a robust electrostatic interaction, corroborating the high stability results (100% for both emulsions).

3.5. Fourier Transform Raman Spectroscopy

Figure 5a shows the complete FT-Raman spectra of cardamomum essential oil and its CNC-stabilized emulsions. FT-Raman is a tool with low peak overlap, reducing the bands' combination and facilitating the correct interpretation of data. In this case, FTIR and FT-Raman were analyzed to better illustrate the possible interactions between nanocellulose and essential oils [43,44].



Figure 5. FT-Raman spectra of cardamomum essential oil and its CNC-stabilized emulsions (**a**) full-spectrum and zoom in the region (**b**) $1350-1900 \text{ cm}^{-1}$, and CNF-stabilized emulsions (**c**) full-spectrum and zoom in the region (**d**) $1350-1900 \text{ cm}^{-1}$.

The pure essential oil showed apparent spectral differences from the emulsions, and its characteristic peaks are presented in Table 3. New peaks appeared after emulsification, and the cardamomum peaks altered their intensity. Figure 5b shows a spectra zoom in the 1250–1900 cm⁻¹.

Peak (cm ⁻¹)	Assignment	Reference	
652	1,8-cineole ring vibration	[22,45]	
755	γ -Terpinene ring vibration	[22,45]	
1446	CH ₂ deformation	[22,45]	
1673	C=C stretching vibrations	[22,45]	

Table 3. Cardamom FT-Raman characteristic peaks and their respective assignments.

Both CNC emulsions showed a new peak shape in the region of $1200-1565 \text{ cm}^{-1}$; this region is associated with C=O vibrations, CH₂ and CH₃ deformations of essential oil, and is also attributed to CH₂, C–C, C–O–H, and C–H bending vibrations of CNCs [46]. This sharp peak is centered at 1404 cm⁻¹. Although both materials have peaks around 1400 cm⁻¹, the literature does not report this Raman shift for either CNCs or essential oil at 1404 cm⁻¹, indicating that a new bond formed between the EO and the solid phase, mainly for sample 30Car–CNC. The peak 1673 cm⁻¹ of cardamonum essential oil increased in intensity for 30Car–CNC and almost disappeared for 20Car–CNC, corroborating the hypothesis of CNC–oil interaction, which forms a strong barrier around the droplets that avoid oil coalescence and the presence of non-bonded oil in the emulsion.

Figure 5c shows the complete FT-Raman spectra of cardamomum essential oil and its CNF-stabilized emulsions. In this case, both CNF emulsions showed very similar spectra, i.e., the emulsification was similar for both systems. As observed for CNC—emulsions' spectra, the EO peak at 1446 cm⁻¹ appeared as a sharp band centered at 1390 cm⁻¹, probably due to CNF–essential oil interactions. Additionally, the peak at 1673 cm⁻¹ was strongly altered, appearing as two peaks centered at 1620 and 1663 cm⁻¹ with a shoulder at 1721 cm⁻¹ (Figure 5d). The CNFs formed a network around the oil droplets that limited their movement and coalescence. This stable gel-like network organized itself around the drops, trapping them inside. For this reason, the characteristic peaks of cardamomum essential oil are less detectable, and cellulose is more intense, since there is a protective CNF layer around the oil droplets, forming a porous structure.

3.6. Nuclear Magnetic Resonance

The NMR spectroscopy investigated molecular interactions, elucidating the supramolecular arrangement architectures. In this case, parameters such as relaxation rate, chemical shifts, molecular diffusion rate, and line width are used in the investigations [47].

The NMR of liquid material is totally dependent on the solubility of the analyzed material in the solvent used, reflecting the distribution and water state in the emulsion. To evaluate the differences in stabilization and, consequently, the solubility effect of EO in stabilized Pickering emulsions, the pure EO was first fully elucidated by NMR. Figure 6 shows the ¹³C spectrum of pristine cardamom essential oil in both deuterated solvents CDCl₃ and D₂O.

The EO has good solubility in CDCl_{3} , presenting a detailed ¹³C spectrum. However, the spectrum obtained with the EO diluted D₂O presented a low-resolution ¹³C spectrum with shifted peaks and some distortion, probably caused by the heterogeneity of the magnetic field caused by a discontinuous liquid phase of the solution EO + D₂O. All peaks in the CDCl₃ ¹³C spectrum also appear in the D₂O spectrum, showing a partial EO solubility in D₂O or H₂O.



Figure 6. ¹³C spectrum of EO Cardamom essential oil (*Elettaria cardamomum*, CAS Number 8000-66-6) diluted with $CDCl_3$ and D_2O .

Figure 7 shows the ¹³C spectra overlay of pure EO (in green), 30Car–CNC (in red), and 20Car–CNC (in blue). It is possible to see the absence of characteristic peaks of α -terpinyl acetate in the emulsions spectra. This might indicate that, after the Pickering emulsion process, the molecule of α -terpinyl acetate migrates to the interior of the micelle formed by the stabilized CNC. Furthermore, in the emulsions spectra, the characteristic peaks found for 1,8-cineole molecules present different shapes, chemical shifts, and resolutions compared to the pure EO, indicating that the molecule has a different interaction with the solvent (D₂O) after the emulsification process. Beyond that, it is possible to verify that the differences in chemical shifts are more evident in carbons 20 and 21, the near atoms of the O atom, and more propitious in interacting with CNC structures and oxygenated solvents D₂O and H₂O.



Figure 7. ¹³C spectra of EO Cardamom essential oil (*Elettaria cardamomum*, CAS Number 8000-66-6), 30Car–CNC and 20Car–CNC emulsions diluted with D₂O.

Figure 8 shows the ¹³C spectra overlay of pure EO (in green), 30Car–CNF (in red), and 20Car–CNF (in blue). Similar to the emulsions stabilized with CNC, the CNF emulsions

presented shifted peaks for the 1,8-cineole molecule. However, the spectra showed characteristic peaks of the α -terpinyl acetate molecule differently than the CNC emulsions. These results indicate that, with CNF structure, even with a gel-like aspect, there is an amount of the EO that is not fully stabilized by the emulsion, being susceptible for the solution and, consequently, phase separation.



Figure 8. ¹³C spectra of EO Cardamom essential oil (*Elettaria cardamomum*, CAS Number 8000-66-6), 30Car–CNF emulsion, and 20Car–CNF emulsion diluted with D₂O.

Thus, the NMR results effectively indicate that the Pickering emulsion process with CNC principally stabilizes the molecule of α -terpinyl acetate, possibly causing it to migrate to the inside of the droplet, while 1,8-cineole, another compound of the cardamom essential oil, presents shifted peaks, indicating interaction with solvent and/or CNC structures, a direct consequence of different solubility. On the other hand, CNF structures did not present the same stability as CNC. Both main molecules found in the pure EO are also found in the emulsion spectrum, indicating phase separation. However, the same shifted peaks for 1,8-cineole are found, showing that this molecule might interact with cellulose structures independently of its crystalline structure. However, considering the oil concentrations used, no significant influence was observed.

3.7. Scanning Electron Microscopy

The 30Car–CNC and 30Car–CNF samples were morphologically evaluated by scanning electron microscopy to examine the nanocellulose organization in the emulsions, as shown in Figure 9. As the emulsions were dried before analysis, it is impossible to identify drops of essential oil, and the nanocellulose showed a tendency to agglomerate due to the hydrogen bonds formed between the nanoparticles. Even with some limitations of the technique, it is possible to verify obvious morphological differences between the samples. The CNC-stabilized emulsions showed a less compact structure than CNF, with pores (identified by the yellow arrows) of different sizes distributed throughout the sample. However, the tendency towards agglomeration made it impossible to identify individual nanocrystals, which can also be associated with the chemical bonds formed between the oil and the CNCs, resulting in a dense structure after drying.



Figure 9. SEM images of 30Car–CNC and 30Car–CNF, a representative scheme of the stabilization mechanism, and the distribution of oil chemical structures (α -terpinyl acetate inside the droplet and 1,8-cineole at the interface) interacting with cellulose.

The CNF emulsion showed a distinct morphology, with a high density of agglomerated nanofibers, as expected, but they were identifiable, in contrast to the CNC emulsion. The spherical voids are attributed to oil droplets' evaporation, and the 3D network formed by the nanofibers is clear, confirming the stabilization mechanism, as illustrated in Figure 9.

4. Conclusions

In this study, cardamom Pickering emulsions stabilized with cellulose nanocrystals or cellulose nanofibers were investigated, concerning cardamom EO's stabilization. CNC emulsions showed smaller droplets and higher absolute surface charges (~60 μ m, -47 mV) than CNF emulsions (160 μ m, -15 mV). The CNC emulsions showed a 34% creaming index and an electrostatic stabilization mechanism, while the CNF emulsions showed 100% stability over 30 storage days, resulting from the CNF's three-dimensional network that entrapped the oil droplets. The emulsions indicated an interaction between nanocellulose, α -terpinyl acetate, and 1,8-cineole active essential oil compounds; where α -terpinyl acetate was "encapsulated", 1,8-cineole was located at the oil–water interface and was available for chemical interaction with cellulose. The interactions resulted in strong solid-phase adsorption, which protects the active oil compounds from the environment and guarantees their

biological activity and physicochemical characteristics, improving cardamom's application in several sectors, such as food, biomedical, cosmetics, and other industries.

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