JOÃO ENRIQUE MASCARENHAS DE FARIA

STUDY ON THE INFLUENCE OF CELLULOSE MICROFIBER IN O/W EMULSIONS STABILIZED WITH TWEEN 80, ETHANOL, AND CASTOR OIL

LAVRAS - MG
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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Engenharia de Biomateriais, para a obtenção do título de Mestre.

Dr. José Manoel Marconcini
Orientador

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ESTUDO DA INFLUÊNCIA DA MICROFIBRA DE CELULOSE EM EMULSÃO O/A ESTABILIZADA COM TWEEN 80 E ETANOL

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Engenharia de Biomateriais, para a obtenção do título de Mestre.

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LAVRAS – MG
2025

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“Amar o perdido
deixa confundido
este coração.

Nada pode o olvido
contra o sem sentido
apelo do Não.

As coisas tangíveis
tornam-se insensíveis
à palma da mão.

Mas as coisas findas,
muito mais que lindas,
essas ficarão.”

Memórias - Carlos Drummond de Andrade

RESUMO

Emulsões são sistemas termodinamicamente instáveis homogeneizados por meio de agentes emulsificantes que intermediam a interação das substâncias imiscíveis. Esses agentes, nos setores da indústria convencional, comumente são sintéticos, causando riscos à saúde humana e meio ambiente. A microfibra de celulose (MFC) é um material abundante, versátil e de baixa toxicidade com propriedades emulsificantes devido a sua capacidade de formar uma rede tridimensional e seu carácter químico anfifílico. No entanto, a sua versatilidade apresenta desafios no uso em emulsões, devido a sua complexidade de interação com materiais diversos. Este trabalho estudou a influência da CMF de marca comercial em sistema óleo-em-água composto por óleo de rícino, Tween 80, etanol e água. O experimento foi delineado por planejamento fatorial e, as formulações, caracterizadas por –análise de estabilidade visual, microestrutura, tamanho de micelas, demanda iônica e estabilidade estrutural. Resultados inferem uma proporção ideal, 25 %, da dispersão de CMF para alcançar uma estabilidade por 7 dias. A sua presença preservou o padrão de formação de micelas provocado pelo Tween 80 e etanol, formando uma macroemulsão, além de atuar como um espessante que, não só modifica, mas intensifica o comportamento viscoelástico, sólido viscoso, a medida que se aumenta o teor de sólidos da CMF de 0,25 % para 0,45%. A demanda iônica mostrou a CMF com um caráter aniônico enquanto o Tween 80 e etanol, não iônico. Conclui-se que a estabilidade foi alcançada devido a formação da rede de CMF no sistema, possivelmente, devido a influência do Tween 80 e etanol sobre a carga superficial da CMF.

Palavras-chave: emulsão de Pickering; microfibras de celulose; demanda iônica; estabilidade estrutural.

ABSTRACT

Emulsions are thermodynamically unstable systems homogenized by emulsifiers, amphiphilic agents that interacts with both immiscible substances. In the industry field, these agents are commonly synthetics, causing risk to human health and environment. The cellulose microfibers (CMF) is an abundant material, versatile and low toxicity with emulsifying properties due to its capacity to form a 3d network and amphiphilic behavior. However, its versatility can be tricking when inserted into an emulsion due to its complex interactions with other materials. This work studied the influence of CMF, from a commercial brand, in an oil-in-water system compound with castor oil, tween 80, ethanol and water. The experiment was designed by factorial design, and the formulations were characterized by visual stability analysis, microstructure, diameter droplets, ionic demand and structural stability. Results have shown an ideal proportion of 25 % CMF dispersion to get stable macroemulsion for 7 days. Its presence preserved the droplets pattern formed by tween 80 and ethanol, making a macroemulsion, besides acting as a thickener that not only modifies, but also intensifies the viscoelastic behavior of the emulsion with the increase of quantity of CMF, from 0.25 % to 0.45 %. The ionic demand showed CMF as an anionic material and tween 80 and ethanol, as non-ionic. The stability was achieved due to the tridimensional CMF network formed by the interaction with the quantities of tween 80 and ethanol.

KEYWORDS: pickering emulsion; cellulose microfibers; ionic demand; structural stability.

INDICADORES DE IMPACTO

O trabalho contribui com as políticas nacionais e internacionais, uma vez que visa o aumento da sustentabilidade e redução dos impactos gerados por surfactantes sintéticos (Tween 80) pela implementação de materiais estratégicos (microfibras de celulose). Essa afirmação se dá pelo desenvolvimento de novos materiais a partir de matéria-prima vegetal, com características potenciais de produção e comercialização. Além disso, contribui na geração de conhecimentos que viabilizam futuras oportunidades de aplicação em indústrias de grande escala, principalmente no setor de cosméticos e na farmacêutica. Diante disso, a pesquisa se alinha aos Objetivos de Desenvolvimento Sustentáveis nas propostas 9, 12 e 13 que se tratam, respectivamente, de transição sustentável para as indústrias, consumo e produção e ação contra as mudanças climáticas. A difusão deste estudo também induz a outras iniciativas, como novas pesquisas de mestrado, doutorado e pós-doutorado do programa de Engenharia de Biomateriais, bem como a outros projetos de formação no âmbito de iniciação científica, cuja expectativa é de que se constituem em novos projetos de investigação-formação-ação. Desse modo, o conhecimento produzido retorna à educação superior e a literatura como um produto gerado no trabalho colaborativo entre universidade e empresa.

IMPACT INDICATORS

The work contributes to national and international policies, as it aims to increase sustainability and reduce the impacts generated by synthetic surfactants (Tween 80) through the implementation of strategic materials (cellulose microfibers). This affirmation is based on the development of new materials from plant-based raw material, with potential characteristics for production and commercialization. Furthermore, it contributes to the generation of knowledge that enables future opportunities for application in large-scale industries, mainly in the cosmetics and pharmaceutical sectors. Given this, the research aligns with the Sustainable Development Goals in proposals 9, 12, and 13, which address, respectively, the sustainable transition for industries, consumption and production, and action against climate change. The dissemination of this study also induces other initiatives, such as new Master's, Doctoral, and Post-Doctoral research within the Biomaterials Engineering program, as well as other training projects in the scope of scientific initiation, whose expectation is that they will constitute new research-training-action projects. Thus, the knowledge produced returns to higher education and literature as a product generated through collaborative work between the university and the company.

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FIRST SECTION

1. INTRODUCTION

Oil-in-water (O/W) emulsions are widely employed in pharmaceutical, cosmetic, and food applications due to their ability to incorporate lipophilic compounds into aqueous media. Nevertheless, they are thermodynamically unstable, with phase separation often occurring through creaming, sedimentation, or coalescence (Costa et al., 2019; Morávková & Stern, 2011; Mussagy et al., 2023; Niroula et al., 2021; Yang et al., 2017). Stability is commonly achieved by adding synthetic surfactants, such as Tween 80, and thickening agents, which lower interfacial tension and increase the viscosity of the continuous phase.

In recent years, cellulose microfibers (CMFs) have gained attention as sustainable stabilizers. Derived from renewable resources, CMFs form three-dimensional networks that immobilize oil droplets and hinder coalescence. They also offer advantages such as biocompatibility, low cost, and the potential to reduce reliance on synthetic surfactants, whose excessive use may raise environmental and toxicological concerns.

The stabilization mechanism of CMFs differs from that of conventional surfactants. Whereas surfactant-based emulsions follow the Hydrophilic–Lipophilic Balance (HLB) concept, CMFs act in a manner similar to Pickering emulsions, where solid particles irreversibly adsorb at the oil–water interface, creating physical barriers to droplet coalescence (Costa et al., 2019; Niroula et al., 2021; Pang et al., 2024; Sanchez-Salvador et al., 2019). This mechanistic duality—HLB-driven stabilization by Tween 80 and Pickering-type stabilization by CMFs—complicates the prediction of emulsion behavior, particularly when both components are combined (Perrin et al., 2020; Yang et al., 2017; Zanin et al., 2002).

Despite these advantages, several factors influence the performance of CMFs in emulsions, including fiber diameter, pH, temperature, ionic strength, and the sequence of addition. Under acidic conditions, CMFs tend to aggregate, impairing stability, whereas alkaline pH enhances dispersion through increased surface charge and can promote synergistic interactions with nonionic surfactants. High CMF concentrations, however, may lead to excessive viscosity, hindering processing (Costa et al., 2019; Dai et al., 2023).

Driven by the demand for sustainable alternatives, current research is focused on partially or fully replacing synthetic surfactants with biomaterials. Within this context, CMFs represent a promising candidate, but their successful application in commercial formulations

requires a deeper understanding of the physicochemical interactions involved. This study addresses this gap by examining how parameters such as order of addition, pH, temperature, and CMF concentration affect the stability of O/W emulsions containing Tween 80, aiming to optimize the use of this biopolymer.

2. OBJECTIVES

2.1. General

Development of an oil-in-water emulsion with CMFs to investigate their stabilization behavior.

2.2. SPECIFICS

- To characterize the commercial CMFs with respect to fiber diameter;
- To stabilize an O/W emulsion using CMFs, Tween 80, ethanol, and castor oil;
- To evaluate emulsion stability through visual inspection and microstructural analysis;
- To investigate CMF interactions with Tween 80 and ethanol by ionic demand analysis;
- To assess the influence of CMFs on emulsion structural stability through rheological testing.

3. LITERATURE REVIEW

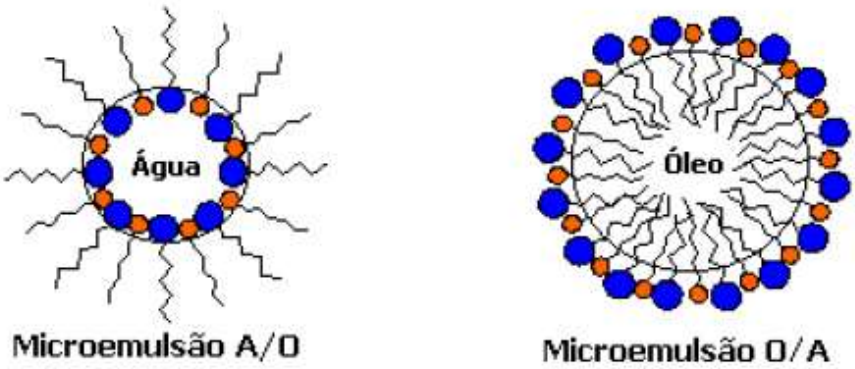
3.1. Emulsions and instability phenomena

Emulsions are dispersed systems in which two immiscible liquid phases—typically oil and water—are combined, with one phase present as droplets dispersed in the other. These thermodynamically unstable systems require external energy for formation and stabilizing agents to preserve their integrity. Their industrial relevance spans pharmaceuticals, cosmetics, and foods, as they enable the simultaneous incorporation of both hydrophilic and lipophilic compounds (Costa et al., 2019; Morávková & Stern, 2011; Niroula et al., 2021; Perrin et al.,

2020; Yang et al., 2017). The stability of emulsions critically depends on factors such as droplet size and distribution, viscosity of the continuous phase, and interfacial properties, which collectively determine shelf life and performance.

Emulsions are generally classified into three main types. Oil-in-water (O/W) emulsions, where oil droplets are dispersed in an aqueous medium, are predominant in lotions and pharmaceutical formulations. Conversely, water-in-oil (W/O) emulsions, with water dispersed in the oil phase, are typical of products such as butter and ointments. More complex systems, such as multiple emulsions (O/W/O or W/O/W), are applied in controlled-release technologies, functioning as reservoirs for active compounds (Huang, 2019; Li et al., 2018; Morávková & Stern, 2011). Each type requires specific emulsifiers, selected according to the desired physicochemical properties of the final product.

Figure 1 – Microemulsion structure illustration



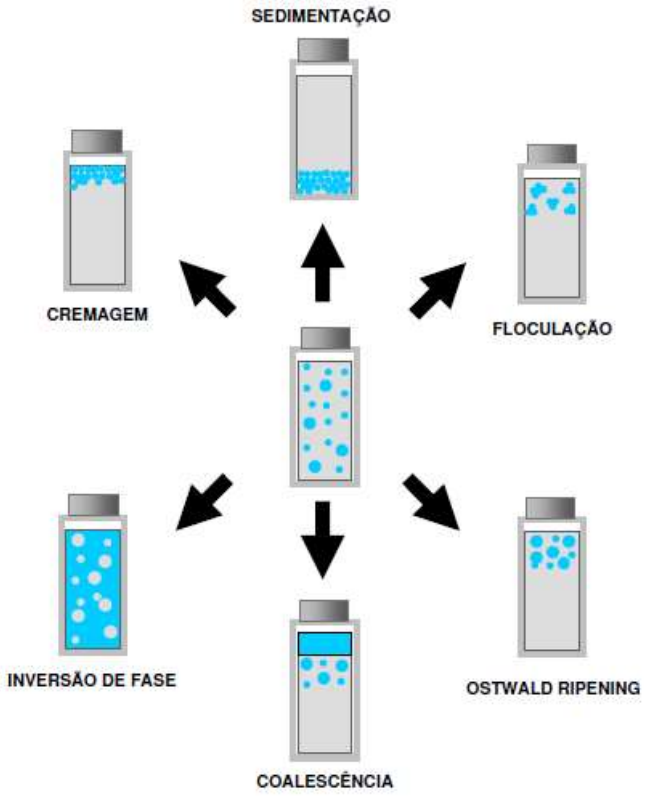
Source: Mollet (2001)

Microemulsions (MEs) are transparent dispersions in which a lipophilic material is distributed in an aqueous medium (or vice versa), stabilized by an emulsifier with or without a suitable co-emulsifier. Unlike conventional emulsions, microemulsions are thermodynamically stable (Franzini, 2006).

The inherent instability of emulsions is manifested through several phenomena driven by the entropic tendency of the system to reduce free energy. Flocculation occurs when dispersed droplets cluster into aggregates, which may or may not induce coalescence. Creaming and sedimentation take place under gravitational forces, depending on the density difference between the dispersed and continuous phases. Ostwald ripening results from the dissolution of smaller droplets (0.1–0.5 μm) into larger ones, increasing average droplet size. Finally, coalescence occurs when two or more droplets merge irreversibly, losing their interfaces and

forming a larger droplet within the dispersed phase (Costa et al., 2019; Huang, 2019; Farias, 2010).

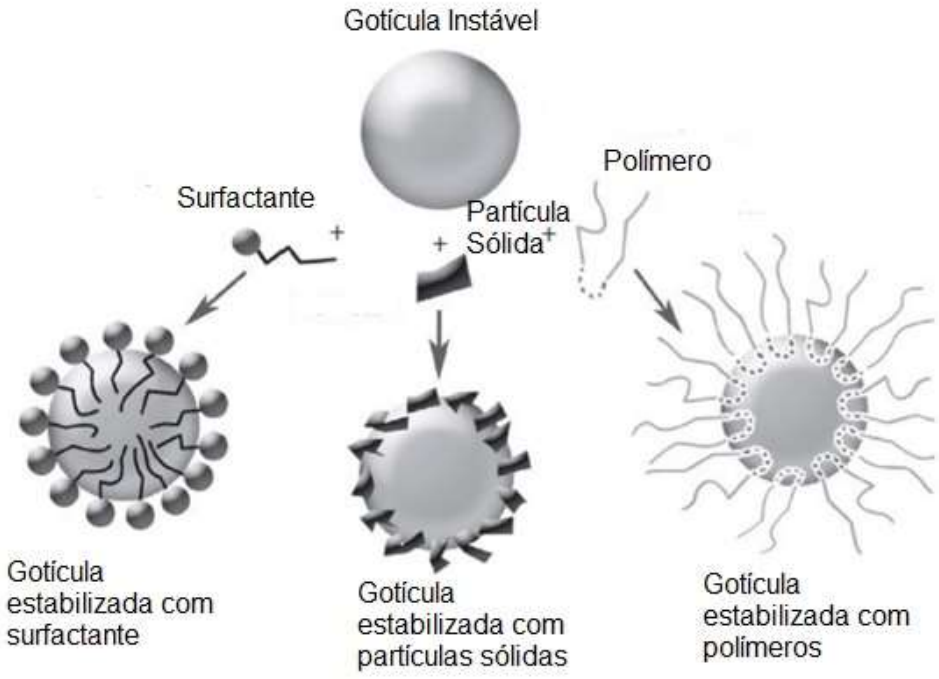
Figure 2 - Representation of visual instability phenomena in emulsions.



Source: FARIAS (2009)

Emulsifiers play a crucial role in stabilizing these systems. Synthetic surfactants such as Tween 80 reduce interfacial tension through their amphiphilic structure, with their performance often described by the Hydrophilic–Lipophilic Balance (HLB) principle. Alternatively, natural emulsifiers such as lecithin or solid particles like cellulose microfibers (CMFs) provide more sustainable options, acting through Pickering stabilization. Additional stabilizers, including polymers and thickening agents, contribute by increasing the viscosity of the continuous phase. Strategic combinations of these agents allow the design of emulsified systems with tailored properties for specific applications, balancing performance, stability, and economic and environmental considerations (Huang, 2019; Rawal et al., 2025).

Figure 3 - Stabilizing interfacial layers formed by surfactants, solid particles, and polymers. Differences in scale were not considered.



Source: Adapted from Costa et al. (2019)

In terms of nomenclature, surfactants and emulsifiers are regarded as distinct classes of molecules, each contributing differently to emulsification. Emulsifiers typically refer to high-molecular-weight polymers and solid particles capable of adsorbing at the oil–water interface, while surfactants are low-molecular-weight amphiphiles. The latter are generally considered the most efficient emulsifying agents due to their rapid diffusion and interfacial activity (Costa et al., 2019; Huang, 2019; Rawal et al., 2025).

3.2. Stabilization Mechanisms: HLB and Pickering

3.2.1. Emulsions based on HLB

The Hydrophilic–Lipophilic Balance (HLB) concept was introduced by William C. Griffin in 1949 as a milestone in emulsion science, providing for the first time a quantitative framework for selecting suitable surfactants. Originally developed for nonionic emulsions, the HLB system assigns numerical values that describe the balance between the hydrophilic and lipophilic portions of surfactant molecules, ranging from 1 (completely lipophilic) to 20

(completely hydrophilic) (Farias, 2010; Franzini, 2006). This innovation revolutionized emulsion development by enabling the prediction of surfactant behavior in different systems.

The HLB theory is grounded in physicochemical principles essential for the formation of stable emulsions. When a surfactant is added to the system, its molecules align at the oil–water interface, with the lipophilic moiety oriented toward the oil phase and the hydrophilic portion toward the aqueous phase. This molecular orientation drastically reduces interfacial tension, facilitating the formation and stabilization of dispersed droplets. The HLB value dictates the type of emulsion formed: surfactants with HLB values between 4–6 are most effective for water-in-oil (W/O) emulsions, whereas values between 8–18 favor oil-in-water (O/W) emulsions. Each specific application, however, requires an optimal HLB value (Farias, 2010; Franzini, 2006).

Tween 80, a widely used polysorbate, exemplifies the practical application of the HLB system. With an HLB value of 15, this nonionic surfactant is highly effective in forming and stabilizing O/W emulsions, making it one of the most frequently employed emulsifiers in pharmaceutical and cosmetic formulations. Its molecular structure, composed of an oleic acid chain (lipophilic) linked to multiple ethylene oxide groups (hydrophilic), provides the ideal balance for systems with a continuous aqueous phase (Wang et al., 2019). The success of Tween 80 is attributed not only to its favorable HLB value but also to its low toxicity and compatibility with a wide range of ingredients.

Despite its utility, formulations relying solely on the HLB concept present significant limitations. The need for relatively high surfactant concentrations to achieve long-term stability can lead to issues such as skin irritation in dermatological applications and environmental concerns related to the persistence of synthetic surfactants. Furthermore, the HLB system does not adequately account for variations in conditions such as temperature, pH, or ionic strength, which can markedly influence surfactant performance. These limitations have driven interest in alternative or complementary stabilization strategies, such as Pickering emulsions, which can reduce or even eliminate the dependence on conventional surfactants (Perrin et al., 2020; Rawal et al., 2025; Yang et al., 2017).

3.2.2. Pickering emulsions

Pickering emulsions represent a major advancement in dispersed system technology, with their foundations established in the pioneering work of Pickering and Ramsden in the early 20th century. This stabilization mechanism differs fundamentally from conventional surfactant-

based systems by relying on solid particles that adsorb irreversibly at the oil–water interface. When particles with intermediate wettability (contact angle close to 90°) are incorporated into the system, they anchor at the interface, forming an efficient physical barrier against coalescence (Pang et al., 2024; Perrin et al., 2020; Rawal et al., 2025; Sanchez-Salvador et al., 2019; Yang et al., 2017). The adsorption energy of such particles can exceed that of traditional surfactants by up to three orders of magnitude, providing exceptional stability that persists even under harsh temperature conditions or prolonged storage.

The advantages of Pickering emulsions are particularly evident when compared to conventional systems, as they exhibit enhanced stability, improved biocompatibility, greater thermal resistance, and the use of sustainable stabilizing agents (Perrin et al., 2020; Yang et al., 2017).

Cellulose microfibrils (CMFs) have emerged as particularly suitable particles for this type of emulsion, combining unique physicochemical properties with environmental benefits. Their elongated morphology and high aspect ratio allow the formation of efficient three-dimensional networks that immobilize oil droplets. The hydroxyl-rich surface offers opportunities for chemical modification, while their renewable origin and biodegradability align with the growing demand for sustainable technologies (Costa et al., 2019; Dai et al., 2023). In practice, these characteristics make CMFs especially valuable for cosmetic and pharmaceutical applications, where safety and performance requirements are critical.

Nevertheless, the use of CMFs in Pickering emulsions is not without challenges. Under acidic conditions (pH below 4), the fibers tend to aggregate, impairing their stabilizing capacity. Similarly, environments with high ionic strength may destabilize the system, limiting their applicability in certain formulations. From a technological perspective, ensuring homogeneous dispersion of the fibers in complex systems and the costs associated with large-scale production remain significant obstacles (Costa et al., 2019; Dai et al., 2023). These limitations highlight the importance of a detailed understanding of particle–phase interactions to optimize system performance.

When compared to HLB-based systems, Pickering emulsions exhibit striking differences. While the HLB approach relies on molecular surfactants that reduce interfacial tension, Pickering stabilization occurs through the formation of physical barriers by solid particles. This fundamental distinction is reflected in practical outcomes: particle-stabilized emulsions generally require lower stabilizer concentrations and demonstrate superior thermal stability, although they are more sensitive to pH variations (Dekker et al., 2023; Deng et al., 2022; Yousufi et al., 2023). The choice between these approaches should consider not only

technical parameters but also cost, regulatory aspects, and market preferences, with a growing trend toward hybrid systems that combine the strengths of both mechanisms.

3.3. Castor oil in cosmetic applications

Castor oil, extracted from the seeds of *Ricinus communis*, is a prominent ingredient in the cosmetic industry due to its remarkable physicochemical properties and biological benefits. Its composition is uniquely rich in ricinoleic acid (approximately 85–90%), a hydroxylated fatty acid that imparts distinctive characteristics to the oil, such as its high viscosity (Mubofu, 2016). This elevated viscosity is particularly advantageous in cosmetic formulations, as it contributes to the formation of an occlusive barrier on the skin surface, minimizing transepidermal water loss and thereby promoting effective hydration (Pées et al., 2009; Shekade et al., 2023; Yeboah et al., 2021). Moreover, the polar nature of ricinoleic acid and its relatively low melting point facilitate its incorporation into a wide range of cosmetic matrices.

In cosmetic applications, castor oil is valued for its emollient, humectant, and conditioning properties. In hair care products such as conditioners and masks, it supports strand strengthening, reduces split ends, and enhances hair shine (Bergfeld et al., n.d.; Singh et al., 2023). For skin care, it is a common component in moisturizers, lip balms, and cleansing products, where it acts to soften and nourish. The hydroxyl group in ricinoleic acid may also influence the oil's interaction with other formulation components, potentially optimizing the solubilization of actives and the overall product stability (Yeboah et al., 2021). Studies further indicate that castor oil possesses antimicrobial and anti-inflammatory properties, which may help reduce skin imperfections and improve smoothness (Azadmard-Damirchi et al., 2011).

However, stabilizing castor oil in emulsion systems, particularly oil-in-water (O/W) emulsions, presents considerable challenges. The high polarity of ricinoleic acid can hinder the formation and maintenance of stable emulsions, as polar oils require more specific emulsifying systems or the addition of co-emulsifiers to achieve the desired stability (Shekade et al., 2023; Yeboah et al., 2021). The intrinsic viscosity of the oil may also complicate the homogenization process during emulsion manufacturing. Instability may manifest as droplet coalescence, flocculation, or phase separation, undermining the product's efficacy and safety over time (Bergfeld et al., n.d.; Shekade et al., 2023). To overcome these obstacles, research has focused on the development of optimized emulsifying systems.

A deeper understanding of the interactions among castor oil, emulsifiers, and other formulation components is therefore crucial for the development of stable, safe, and high-performance cosmetic products.

3.4. Emulsifiers

3.4.1. Polissorbate 80 (Tween 80)

Tween 80, chemically known as polysorbate 80 ($C_{64}H_{124}O_{26}$), is a nonionic surfactant widely used in the pharmaceutical, cosmetic, and food industries. Its molecular structure consists of an anhydrosorbitol core esterified with oleic acid (a C18:1 fatty acid) and polymerized with approximately 20 ethylene oxide units. This configuration provides Tween 80 with a unique balance between its hydrophilic (polyoxyethylene chains) and lipophilic (oleic acid tail) portions, resulting in a Hydrophilic-Lipophilic Balance (HLB) value of 15, which makes it particularly effective in stabilizing oil-in-water (O/W) emulsions. Interfacial tension studies have demonstrated that Tween 80 significantly reduces oil–water interfacial tension, depending on concentration, thereby facilitating the formation and maintenance of droplets (Nazar et al., 2009; Nielsen et al., 2016).

The emulsifying mechanism of Tween 80 is based on three main physicochemical principles. First, its amphiphilic nature allows for oriented adsorption at the oil–water interface, with the oleic acid chain embedded in the lipophilic phase and the polyoxyethylene chains extended into the aqueous phase. Second, the hydrated polyoxyethylene chains create a steric barrier that prevents droplet approach and coalescence. Finally, at concentrations above the critical micelle concentration, Tween 80 forms micelles that act as molecular reservoirs, maintaining interfacial saturation during thermodynamic fluctuations (Nielsen et al., 2016).

3.4.2. Ethanol

The addition of ethanol to the aqueous phase consistently leads to a reduction in the interfacial tension between the oil and water phases. This reduction is a fundamental principle in emulsion formation, as it directly decreases the energy required to create and stabilize a new interfacial area during the emulsification process. For instance, at specific concentrations—around 40% ethanol in the continuous phase of emulsions with sunflower oil—the influence of

ethanol on interfacial tension becomes prominent, suggesting a dominant effect. This phenomenon is hypothesized to result from ethanol's ability to penetrate and modify the surfactant monolayer at the interface, thereby altering its optimal curvature and more effectively reducing the interfacial free energy than the primary surfactant alone (Ferreira et al., 2020). This indicates a direct molecular-level interaction of ethanol in lowering the surface free energy differential.

Ethanol also significantly modulates surfactant solubility within the aqueous phase, which in turn determines its partitioning behavior and effective concentration at the oil–water interface. For example, at high ethanol concentrations, certain surfactants such as lecithin may exhibit reduced solubility, leading to aggregation or precipitation and a consequent loss of emulsifying efficiency. Similarly, nonionic surfactants such as Tween 20 and Tween 80 may become excessively soluble in the ethanol-rich continuous phase, resulting in their displacement from the interface and a decrease in stabilizing capacity (Ferreira et al., 2020).

3.5. CMF as a natural emulsifier

Droplets in emulsions typically range in size from 100 nm to 100 μm, with emulsions generally classified as either oil-in-water (O/W) or water-in-oil (W/O) (Costa et al., 2019; Jutakridsada et al., 2020; Shkreli et al., 2023; Terescenco et al., 2020). Immiscible liquids are mixed with the aid of a third agent with emulsifying function, which associates the polar and nonpolar phases. Immiscibility, whether partial or complete, arises from thermodynamic instability caused by the large differences in surface tension between the phases involved. The primary strategy for stabilizing such mixtures is the use of emulsifying agents that create an energetic barrier at the interface of the two phases due to their amphiphilic nature—that is, their ability to interact with both polar and nonpolar materials—thus preventing coalescence (Costa et al., 2019; Moravkova & Filip, 2013; Perrin et al., 2020; Terescenco et al., 2020). Within this context, cellulose emerges as a promising biomaterial for stabilizing thermodynamically unstable emulsions.

The interfacial barrier can be achieved through the adsorption of a stabilizing layer composed of amphiphilic surfactants or surface-active agents, polymers, or solid particles (Costa et al., 2019; Perrin et al., 2020; Winuprasith & Suphantharika, 2015). Surfactants, which are typically amphiphilic molecules consisting of both polar and nonpolar moieties, are effective emulsifiers but may also cause adverse effects on human health and the environment,

leading to consumer aversion in favor of natural product alternatives (Perrin et al., 2020). Consequently, research has increasingly focused on the development of non-toxic and environmentally sustainable biomolecules as alternative emulsifying agents to conventional surfactants.

As previously mentioned, cellulose exhibits amphiphilic properties due to its structural anisotropy, which enables its function as a solid emulsifier suitable for forming Pickering emulsions. Parameters such as particle shape, wettability, size, concentration, and interparticle interactions must be carefully considered to fully understand emulsions stabilized by solid particles (Winuprasith & Suphantharika, 2015). Given that this study aims to investigate the emulsifying properties of commercially available microfibrillated cellulose (CMF) for stabilizing emulsions, accounting for these parameters will be essential to accurately describe its effects.

Cellulose is the most abundant biomolecule on Earth and can be sourced from a wide range of natural feedstocks, making it a strategic material for industrial applications due to its renewability, sustainability, biodegradability, and non-toxicity (Winuprasith & Suphantharika, 2015). The CMF used in this study is a commercial-grade material with 90% purity, derived from eucalyptus. Therefore, characterization of this material will be indispensable to better understand its effects in the formulations in which it will be incorporated.

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SECOND SECTION – ARTICLE

Study on the Influence of Cellulose Microfiber in O/W Emulsions Stabilized with Tween 80, Ethanol, and Castor Oil

1. INTRODUÇÃO

Emulsions are thermodynamically unstable systems composed of two or more immiscible liquid phases, such as oil and water, in which one phase is dispersed within the other as droplets. In the cosmetics and personal care industry, they are essential, ranging from prolonged-release fragrances to skin moisturizers and vitamin delivery systems. Traditionally, synthetic surfactants, such as Polysorbate 80 (Tween 80), have been employed to promote homogenization. However, concerns regarding the adverse effects of these compounds—including skin irritation, allergies, and potential disruption of the microbiota—have driven the search for safer and more sustainable alternatives. This shift directs research toward naturally derived materials, which offer notable advantages such as greater biodegradability, lower toxicity, biocompatibility, and potential antimicrobial activity, in line with the growing demand for “clean-label” products (Costa et al., 2019; Nielsen et al., 2016).

Pickering emulsions represent a promising alternative to conventional systems, being stabilized by finely divided solid particles instead of surfactant molecules. In these emulsions, organic or inorganic micro- and nanoparticles irreversibly adsorb at the oil–water interface, forming a robust mechanical barrier that physically prevents droplet coalescence (Deng et al., 2022; Perrin et al., 2020). This mechanism provides superior stability compared to classical emulsions (Dekker et al., 2023).

Microfibrillated cellulose (CMF), derived from abundant renewable resources and produced through mechanical treatments, exhibits properties such as micro- and nanoscale dimensions, biodegradability, biocompatibility, renewability, high mechanical strength, and a high degree of crystallinity. These characteristics enable CMF to form a cohesive three-dimensional network, effectively encapsulating oil droplets and preventing their coalescence (Costa et al., 2019; Foster et al., 2018; Gouzy et al., 2019; Xhanari et al., 2011).

Its emulsifying capacity is directly proportional to the amount used and its wettability, allowing precise control over emulsion properties (Winuprasith & Suphantharika, 2013, 2015). Emulsions stabilized by CMF display stability against gravity-induced sedimentation and, most

importantly, against coalescence. Its biodegradable and non-toxic nature is advantageous for the cosmetics industry, aligning with the growing consumer preference for natural and sustainable ingredients (Franca & Ueno, 2020). Additionally, CMF can act as a rheological modifier, increasing the apparent viscosity of the continuous phase, which is valuable for product texture and phase separation prevention, thereby contributing to the overall structural integrity of the emulsion (Iotti et al., 2011; Xu et al., 2018).

Despite its potential, the incorporation of microfibrillated cellulose in emulsified systems faces challenges that must be overcome for large-scale industrial application. In this context, the present work aims to incorporate commercial CMF into O/W emulsions and evaluate its influence on stabilization through analyses of visual stability, microstructure and droplet size, structural stability, and ionic demand.

2. METHODOLOGY

2.1.1. Materials and CMF characterization

2.1.1.1.1. Materials

Castor oil (97–103%, pH 6.6–6.8) was obtained from ACS Científica, absolute ethanol (99.8%) from Êxodo Científica, and Polysorbate 80 (Tween 80) from Dinâmica Química Contemporânea Ltda.

2.1.1.1.2. CMF characterization

Sample preparation and visualization were carried out at the Electron Microscopy Laboratory (LME), in the Phytopathology Department of the Federal University of Lavras (UFLA). The CMF samples were prepared by dispersing the commercial-grade material (90% purity) at a concentration of 0.001%. Triplicate aliquots of the dispersion were pipetted onto aluminum foil–covered metallic stubs, stored in a desiccator overnight, and subsequently coated with a thin layer of gold using a metal evaporator (model SCD 050). The metallized samples were then analyzed using a STEM-FEG scanning electron microscope (model CLARA – TESCAN, Oberkochen, Germany) to obtain high-resolution micrographs of surface

morphology. The acquired images were processed with ImageJ software, from which fiber diameters were extracted to perform a descriptive analysis of diameter distribution. Also the solid content was measured following TAPPI T 240 method.

2.1.2. Preparation of formulations

For data analysis, a full two-level factorial design was employed with one or two variables depending on the formulation. Three controls were defined: one stabilizing castor oil with Tween 80 and ethanol only, another using CMF alone, and a third combining Tween 80, ethanol, and CMF. Parameters such as castor oil concentration (5%), temperature (25 °C), pH (8), stirring speed (9,000 rpm + 14,000 rpm), and stirring time (10 min) were kept constant. Table 1 presents the adopted proportions, while Table 2 details the factorial design matrix with coded variables.

Table 1 - Proportions of materials proposed for investigation

Variáveis	**Code**	**Levels**	
Tween:Ethanol	T:E	50%	70%
Cellulose Microfiber	CMF	0,45%	0,25%

Source: from autor (2025)

2.1.2.1.1. Tween 80 and ethanol

To investigate the effects of CMF on emulsion stabilization, a control formulation was designed with distilled water, castor oil, Tween 80 (a commercially available synthetic surfactant), and ethanol (99.5% purity).

Oil-in-water (O/W) emulsions were then prepared by fixing the castor oil concentration at 5% and varying Tween 80:ethanol ratios (T:E = 1:2) between 50% and 70%. Each formulation had a total mass of 10 g, and ingredients were placed in a 50 mL beaker. Following preliminary tests and with modifications from Nazar et al. (2009), the optimal Tween 80:ethanol ratio was determined to be 1:2. The mixtures were homogenized using an Ultra Turrax homogenizer (IKA, model T-18, with S 18 N–19 G disperser) for 10 minutes—1 minute at 8,000 rpm followed by 9 minutes at 14,000 rpm. The first minute began with castor oil mixed

with T:E emulsifiers, and water was added after a few seconds of agitation. pH (basic, >7) and temperature (25 °C) were controlled.

Table 2 - Full factorial design matrix with coded variables.

Samples	T:E [% w/w.]	CMF [% s.c.]*	CMF [% w/w]**
1	50	0	0
2	70	0	0
3	0	0,45	95
4	0	0,25	95
5	50	0,45	45
6	50	0,25	45
7	70	0,45	25
8	70	0,25	25

*s.c. – solids content;

** percentage of CMF dispersion

Source: From autor (2025)

Each formulation was visually classified as opaque, partially opaque/translucent, translucent, with apparent oil droplets, coalescent, or flocculated. Table 3 lists the tested formulations.

Table 3 – Combination of the mass quantity between castor oil, Tween 80, ethanol and distilled water formulated for the study.

Samples	1	2
Oil [g]	0,5	0,5
***T:E* [g]**	5	7
Water [g]	4,5	2,5

Source: from autor (2025)

2.1.2.1.2. CMF + castor oil

CMF dispersions in water were prepared at two concentrations, 0.47% and 0.26% solids, with pH adjusted to >7 using pH strips.

Formulations were prepared following the same procedure as for the Tween 80:ethanol system. Each was stored in test tubes and evaluated under backlighting to classify their appearance as opaque, partially opaque/translucent, translucent, with visible oil droplets, coalescent, or homogeneous. The adopted proportions are shown in Table 4.

Table 4 - Combination of quantity and mass between CMF dispersion, castor oil and distilled water formulated for the study.

Sample	**3**	**4**
1% CMF [g]	5,5	2,5
Oil [g]	0,5	0,5
Water [g]	4	7

Source: From autor (2025)

2.1.2.1.3. Tween 80, ethanol and CMF

A ternary formulation was then prepared with 5% castor oil, Tween 80:ethanol emulsifiers, and CMF. The oil phase (T:E + castor oil) was first homogenized, followed by the addition of CMF dispersion after a few seconds, in accordance with the control protocol. pH was adjusted to >7 at each stage using pH strips, with final adjustments performed after mixing. Temperature was maintained at 25 °C using a heating plate. Table 5 summarizes the tested formulations.

Table 5 - Mass quantity combinations of CMF, T:E and castor oil dispersion formulated for the study.

Sample	**5**	**6**	**7**	**8**
CMF [% s.c.]*	0,45	0,25	0,45	0,25
CMF [g]**	4,5	4,5	2,5	2,5
Oil [g]	0,5	0,5	0,5	0,5
T:E [g]	4	4	7	7

* amount of CMF in % solids content.

** amount of CMF dispersion in mass.

Source: From autor (2025)

2.1.3. Analysis of visual stability, microstructure and determination of droplet diameter

The emulsions were subjected to visual stability analysis, focusing on phase separation and microstructural variation, i.e., the number, size, and shape of dispersed oil droplets per microscopic slide. Stability was assessed at room temperature over 7 days.

An optical light microscope was used to evaluate emulsified droplets. One droplet was pipetted onto a glass slide, and droplet size and distribution were analyzed using ImageJ, with a minimum count of 100 micelles.

2.1.4. Ionic demand analysis

In colloidal systems, it is essential to understand how ionic dynamics and intermolecular forces interact with suspended particles, as this determines macroscopic stabilization. For example, in aqueous media, cellulose fibers—rich in hydroxyl groups—can dissociate depending on pH, generating negative surface charges. The interaction between this surface charge and surrounding ions promotes either attraction or repulsion phenomena (Marques, 2013). Such interactions directly affect energy distribution within the system, enhancing dispersion homogeneity and preventing instability phenomena such as coalescence, flocculation, and sedimentation.

In this study, this methodology was applied to compare ionic demand behavior between control systems and formulations containing CMF. Measurements were performed in triplicate across four timepoints over one week. Prior to each measurement, emulsions were manually shaken vertically to redisperse internal material. Ionic demand (μeq/L) and initial potential (mV) were determined using a CAS-II touch! charge analysis system (Emtec Electronic GmbH, Leipzig, Germany), based on titration principles to predict and understand interactions between CMF and T:E.

2.1.5. Viscoelastic characterization: rheology

Rheology, the study of flow and deformation of matter, is particularly relevant for emulsified systems, which are viscoelastic materials capable of displaying a wide range of flow behaviors due to intermolecular interactions and microstructural organization (Huang, 2019).

The flow behavior of a material lies on a spectrum between the extremes of a solid and a liquid. Elastic solids can recover their original structure after shear stress, whereas viscous liquids deform continuously under shear stress and retain the deformation after stress removal (Huang, 2019).

Two key parameters describe material behavior: the elastic modulus (G′) and the viscous modulus (G″), both expressed in pascals (Pa). G′ represents stored energy during oscillatory deformation (storage modulus), while G″ represents dissipated energy due to viscous friction (loss modulus). Materials exhibiting solid-like behavior (viscoelastic solids) typically show G′ > G″, indicating energy storage dominance, whereas viscous liquid–like materials exhibit G″ > G′, indicating structural dissipation. If G′ = G″, the material behaves as both solid and liquid. If G″ = 0 and G′ ≠ 0, the material is purely elastic; conversely, if G′ = 0 and G″ ≠ 0, it is purely viscous (Huang, 2019).

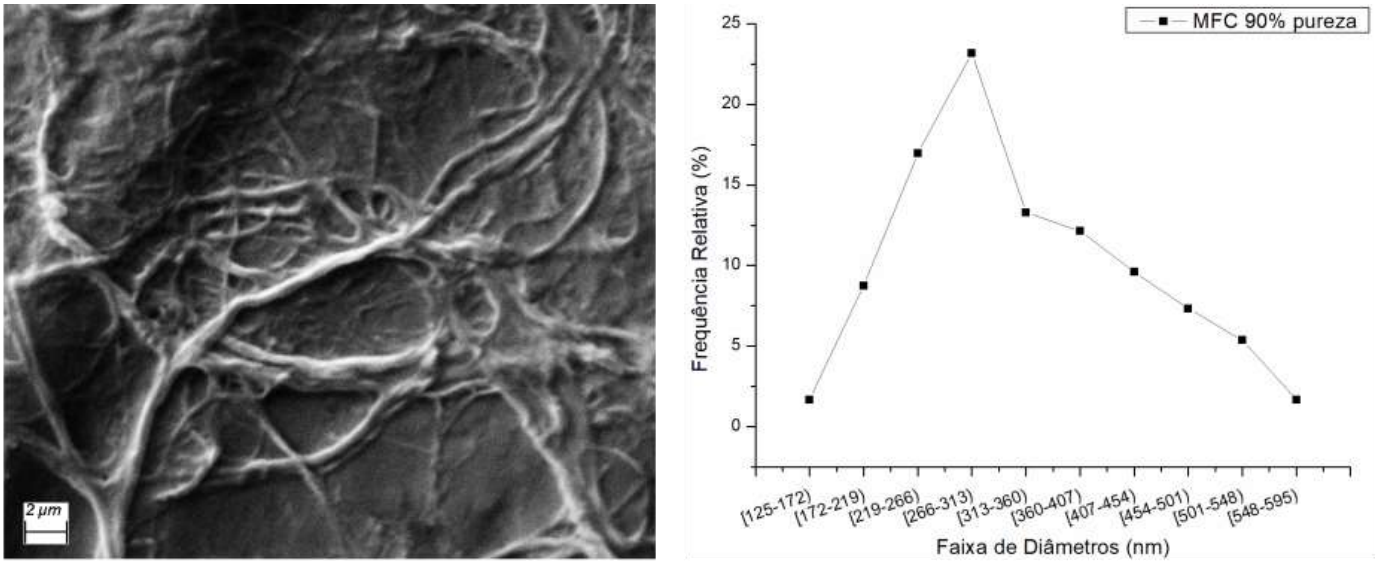
Oscillatory amplitude and frequency sweep tests were applied to evaluate the influence of CMF in combination with other ingredients on the flow behavior of the final formulation. A parallel plate geometry (50 mm, 1 mm gap) was employed. Frequency sweeps were performed from 0.001% to 100% shear stress at 1% shear strain, while amplitude sweeps covered 0.01% to 100% shear strain at 1 Hz angular frequency, adapted from Iotti et al. (2011). Rheological measurements were carried out using an Anton Paar MCR 102e rheometer.

3. RESULTS AND DISCUSSIONS

3.1.1. CMF characterization

The solid content was measured in triplicate and the average value was 3,54%. Scanning electron microscopy (SEM) enabled visualization of cellulose microfibers with a purity of 90% (Figure 4), allowing measurement of fiber diameter and characterization of fiber variability.

Figure 4 - Scanning electron microscopy image of 90% CMF on the left; fiber diameter distribution graph on the right.



Source: From autor (2025)

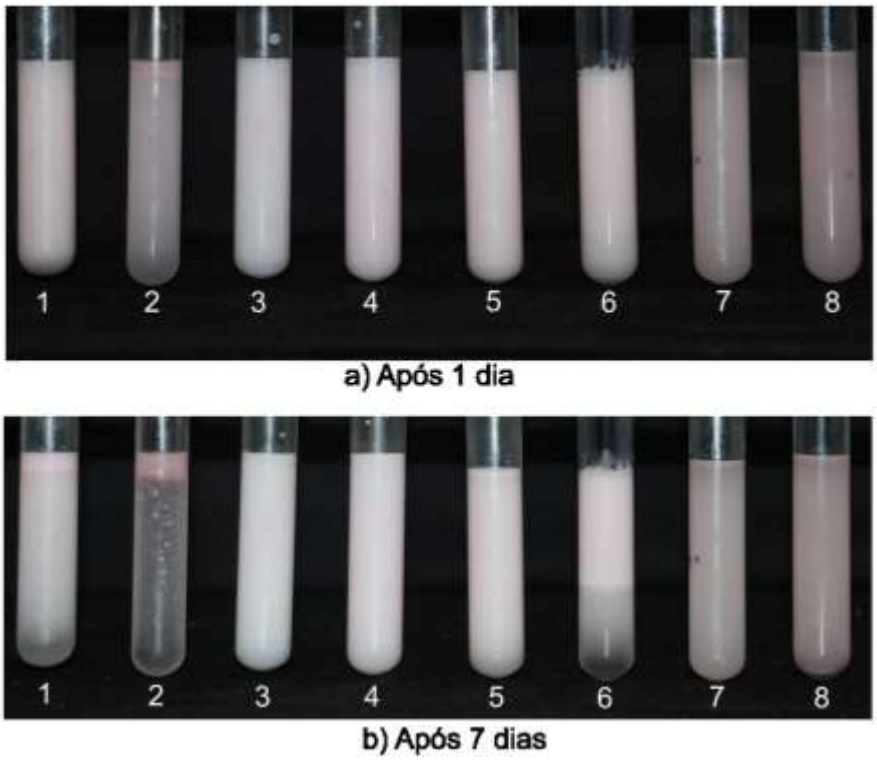
The images show that the fibers intertwine in multiple directions, hindering the identification of the start and end points of each fiber and thus preventing length measurement, restricting analysis to fiber diameter only. Statistical analysis revealed that the fibers display varied diameters in the micrometer range.

The frequency distribution of fiber diameters indicated that most fibers fall within the range of 127 nm to 593 nm, with a mean of 333 nm and a standard deviation of 98 nm. Therefore, they can be considered micrometric, since nanomaterials are typically defined as having dimensions between 1 nm and 100 nm (Foster et al., 2018). The diameter distribution curve indicates a higher density of fibers within the range of 266–313 nm. Fiber diameter is a key parameter, as it influences the physicochemical behavior of fibers when interacting with other molecules in different applications, such as the production of colloidal composites in emulsified systems (Costa et al., 2019).

3.1.2. Visual stability

Visual stability was evaluated through photographic images taken one and seven days after emulsification. Figure 5 shows the samples after one day (5a) and after seven days (5b) of storage at room temperature in late June 2025.

Figure 5 - Image of emulsions 1 and 7 days after preparation.



Source: From autor (2025)

Emulsified systems with surfactants (samples 1 and 2) exhibited coalescence and creaming after the first day. The color of sample 2 suggests a change in turbidity, indicating microemulsion formation. Samples 3 and 4 formed a thin oil layer on the surface, suggesting coalescence. Formulations 5 and 6 were stable one day after preparation but destabilized after seven days, with evidence of creaming. Finally, samples 7 and 8 remained stable over the seven-day period. The clear region observed at the bottom of tube 8 may result from water released due to droplet creaming, or from entrapped air, as also observed by Aaen et al. (2019). The reddish coloration arises from the presence of a small amount of Nile Red, a fluorochrome used to contrast castor oil under epifluorescence optical microscopy for future applications.

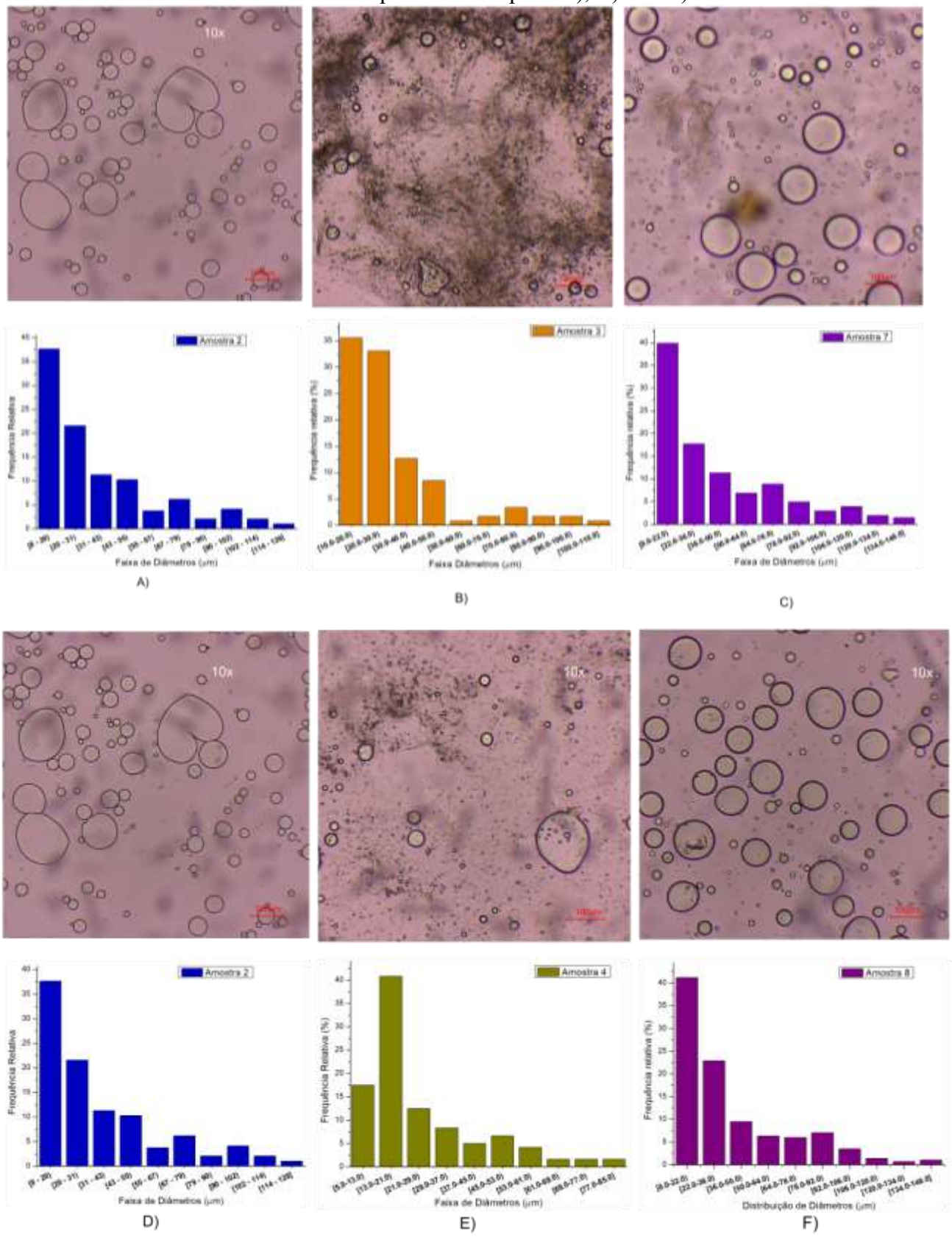
The apparent stability of samples 7 and 8 compared to controls 2, 3, and 4, and the instability of samples 5 and 6 compared to controls 1, 3, and 4—under identical preparation conditions—suggest that the proportional fraction of CMF dispersion influences emulsion stability independently of concentration, i.e., CMF solids content. This finding suggests the

existence of an optimal proportion of CMF dilution for stabilizing the system. Further investigation is warranted regarding the effect of solids content in the final formulation.

3.1.3. Microstructure analysis and droplets diameters distribution

The morphology and droplet diameter distribution of control emulsions and those containing CMF were analyzed on the day of preparation. Since samples 5 and 6 displayed instability in visual inspection, their microstructure and droplet size distribution were excluded, as was sample 1. The following figures present microscopic characterization of emulsions and their corresponding diameter distribution graphs relative to frequency for samples 2, 3, and 7, and 2, 4, and 8. ANOVA and Tukey's test were applied to compare samples, with results available in Appendix I.

Figure 6 - Microscope images and droplet diameter distribution graph in a comparative sequence of samples. A), B) and C).



Source: From autor (2025)

Microscopy images (Figure 6) and droplet diameter distributions revealed that samples displayed polydisperse behavior, with droplet sizes ranging from 8 µm to 150 µm, classifying them as macroemulsions (Kurpanik et al., 2022; Sun et al., 2017). Distribution analysis was performed manually using ImageJ to extract droplet populations for each sample. Control samples 2 (7 A/D), 3 (7 B), and 4 (7 E) demonstrated characteristic and distinct behaviors regarding both droplet count per slide and predominant diameter ranges. Table 6 presents mean values, standard deviations, and maximum and minimum diameters (µm).

Table 6 - Comparison between the characteristic variables for each sample showing the mean, maximum and minimum values, standard deviation and sample size.

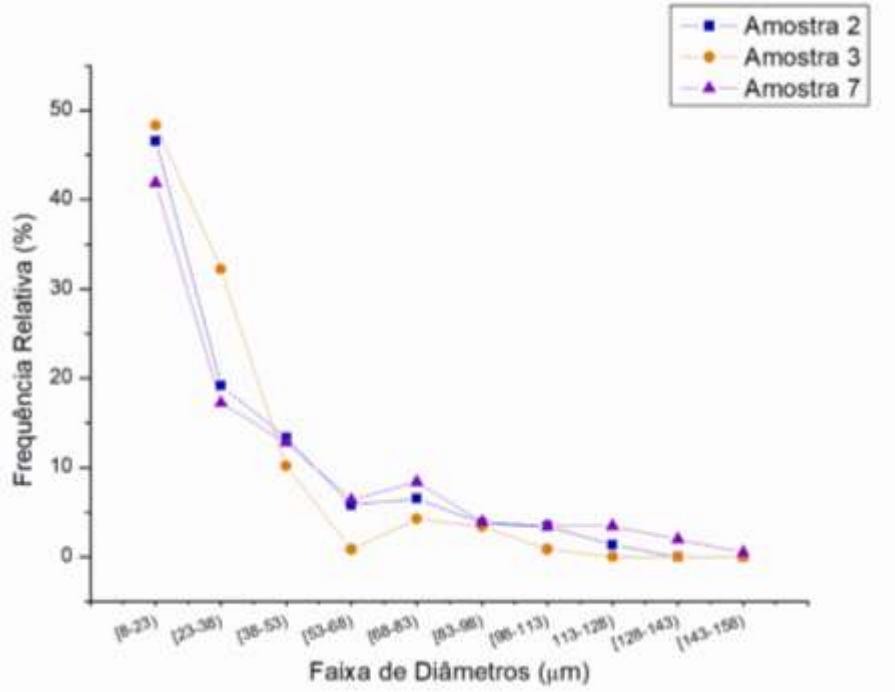
Samples	Average (µm)	Max. (µm)	Min. (µm)	D.P. (µm)	Size (N)
2	$36,68 \pm 1,56_{a*}$	126,4	8,31	26,72	292
3	$30,37 \pm 1,81_{ab}$	109,42	11,33	19,71	118
4	$25,77 \pm 1,66_{b}$	78,44	6,9	18,17	120
7	$42,56 \pm 2,33_{ac}$	145,97	9,8	33,22	203
8	$38,81 \pm 1,77_{ac}$	148,22	9,57	29,84	284

*Averages followed by same letters are not statistically different by Turkey test.

Source: From autor (2025)

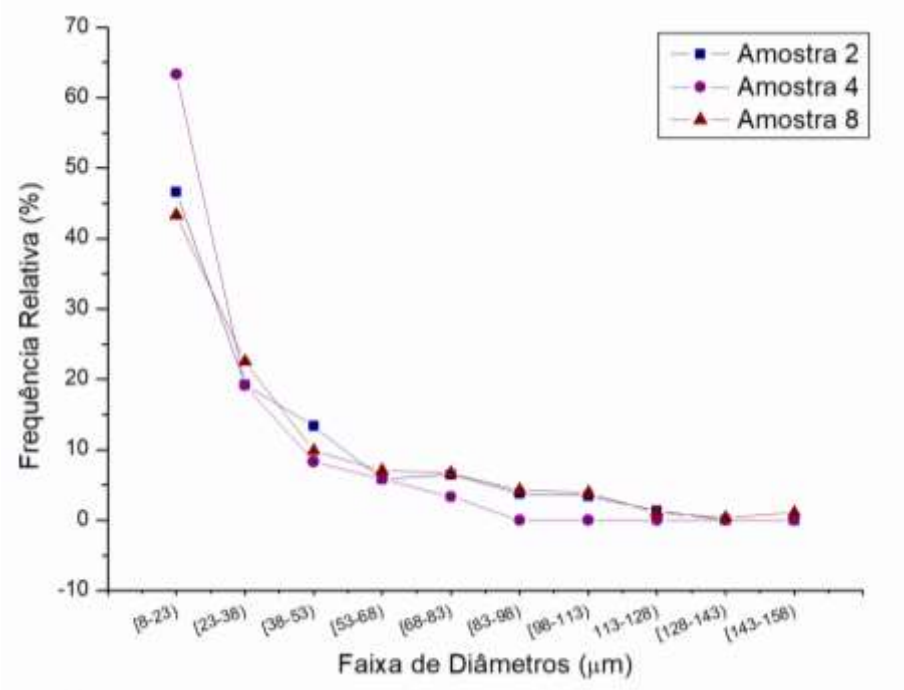
Figure 7 graphically compares samples 2, 3, and 7, where samples 2 and 3 serve as controls and sample 7 corresponds to the surfactant–CMF combination. All three exhibit right-skewed diameter distributions, with medians of 24.4 µm (2), 23.94 µm (3), and 29.4 µm (7). The highest density of droplet frequency lies between 8–23 µm, accounting for 46.58%, 48.31%, and 41.87% of droplets, respectively, although sample 7 shows greater diameter diversity. Comparisons 2–3 and 2–7 (Table 6) showed no significant differences, whereas 3–7 did.

Figure 7 - Comparative graph of the behavior of the diameter distribution of samples 2 - 3 - 7.



Source: From autor (2025)

Figure 8 - Comparative graph of the behavior of the diameter distribution of samples 2 - 4 - 8..



Source: From autor (2025)

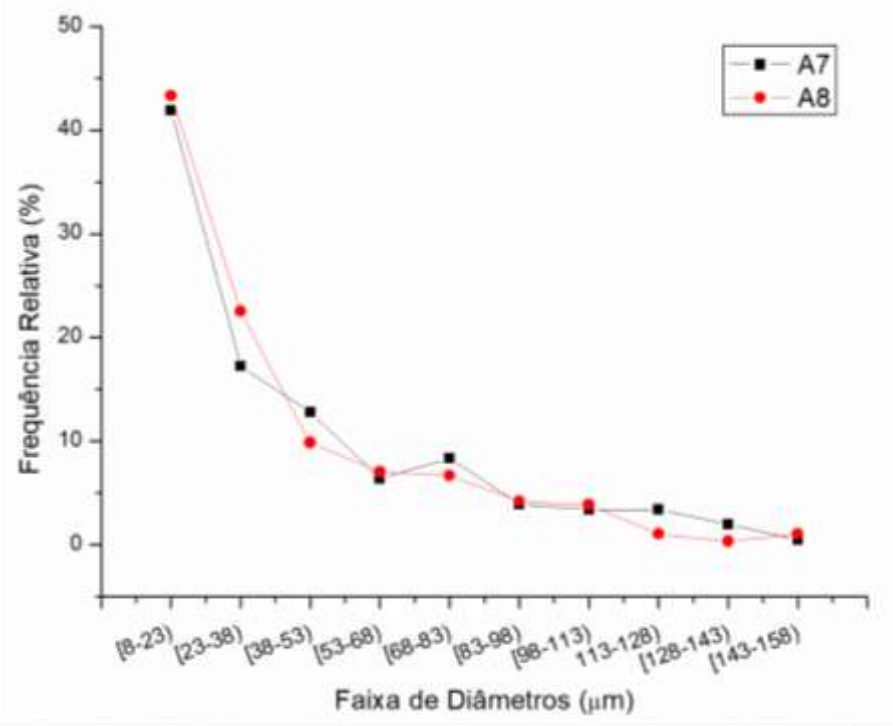
Figure 8 compares samples 2, 4, and 8, also showing right-skewed distributions with medians of 24.4 μm (2), 18.83 μm (4), and 26.15 μm (8). As before, the highest density of

droplets lies between 8–23 μm, accounting for 46.58%, 63.33%, and 43.31% of droplets, respectively. Sample 8, containing the CMF–surfactant interaction, exhibited greater diameter diversity. Comparisons 2–8 showed no significant differences, whereas 2–4 and 4–8 did.

Samples 7 and 8 presented microstructural patterns similar to control groups, indicating a synergistic interaction between Tween 80, ethanol, and CMF under the tested physical conditions. Comparison of these two samples (Figure 9; Table 6) indicates that CMF concentration did not significantly affect micelle diameter. However, microscopic images suggest that dispersed phase micelles are more uniformly spherical in CMF-containing samples compared to controls.

This interaction may arise from the dual mechanisms of emulsification: CMF fibers form a supportive network aiding droplet formation and dispersion during pre-homogenization, while surfactants stabilize via hydrophilic–lipophilic balance. The CMF-stabilized barrier is known as Pickering emulsification, whereas the latter corresponds to conventional emulsification (Perrin et al., 2020; Winuprasith & Suphantharika, 2015).

Figure 9 - Comparative graph of the behavior of the diameter distribution of samples 7 – 8.

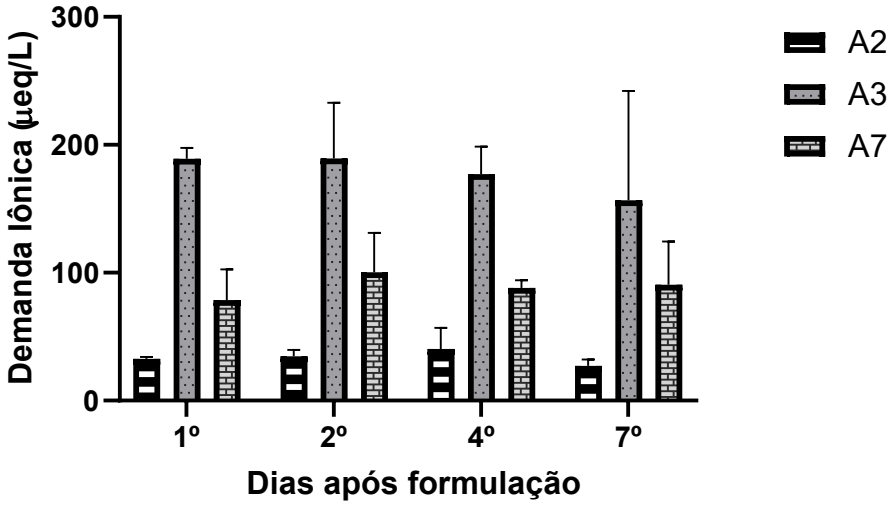


Source: From autor (2025)

3.1.4. Ionic demand analysis

Ionic demand was measured for the most stable sample sequences, namely 2–3–7 and 2–4–8. ANOVA, performed using GraphPad Prism 8.4.2, detected no significant differences across days analyzed, suggesting ionic stability over the seven-day period.

Figure 10 - Comparative histogram of ion demand between samples 2 - 3 - 7 over seven days of observation.



Source: From autor (2025)

In sequence 2–3–7 (Figure 10), ionic demand decreased between samples 3 and 7, with significant differences on days 1 and 4, but not on days 2 and 7. Large standard deviations on days 2 and 7 suggest possible interference from impurities or non-uniform dispersion. Between samples 2 and 7, ionic demand showed a slight increase, though not statistically significant in Dunnett's test ($p > 0.05$).

In sequence 2–4–8 (Figure 11), ionic demand decreased between samples 4 and 8, with significant differences on days 1, 2, and 7, but not on day 4. Between samples 2 and 8, demand slightly increased, reaching significance on days 1 and 7, likely for reasons similar to the 3–7 comparison.

Comparing samples 7 and 8 (Figure 12) revealed only a slight difference in ionic demand, statistically insignificant. These results indicate that CMF substantially reduces surface charge in the presence of Tween–ethanol, behaving as an anionic material (Signori-Iamin et al., 2024), whereas Tween 80 and ethanol are nonionic (Nogueira et al., 2022; Ribeiro et al., 2024; Shaker et al., 2017). Appendix II provides further details of ionic demand analyses.

Although castor oil ionic properties were not tested, literature confirms its nonionic behavior (Chauke et al., 2019; Fredrick et al., 2023; Panhwar et al., n.d.).

Figure 11 - Comparative histogram of ion demand between samples 2 - 4 - 8 over seven days of observation.

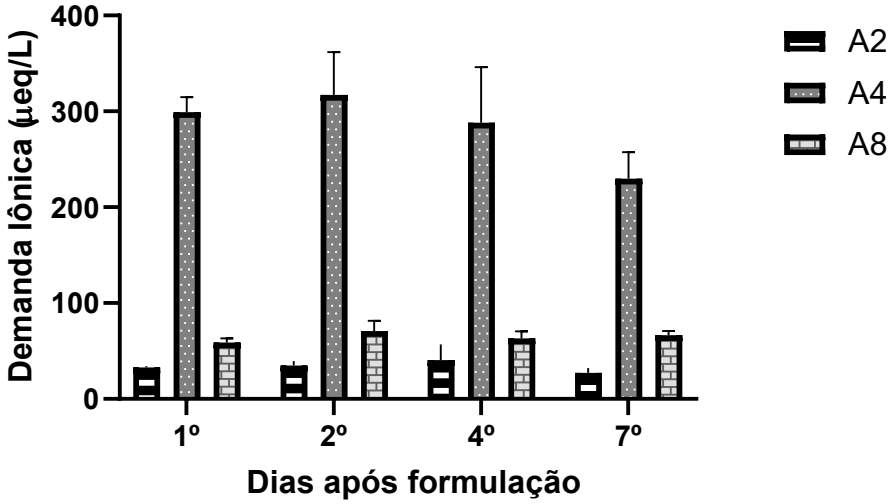


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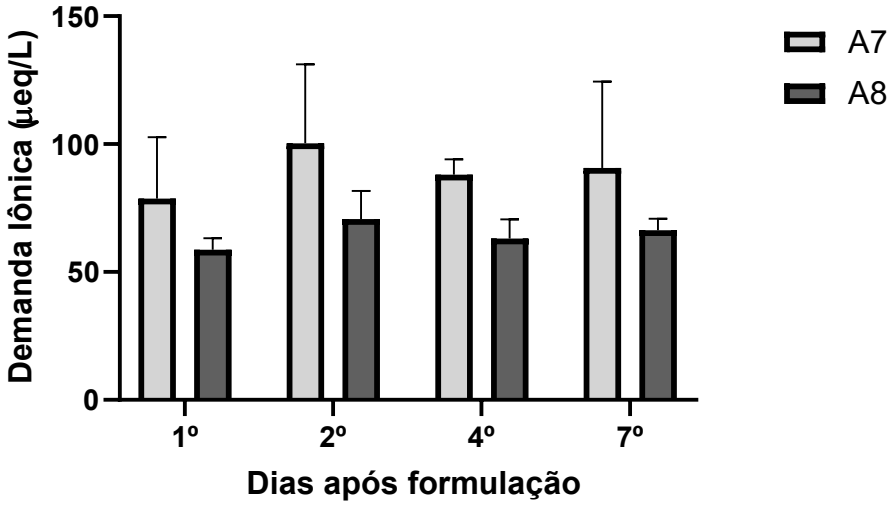
Figure 12 - Comparative histogram of ion demand between samples 7 - 8 over seven days of observation.



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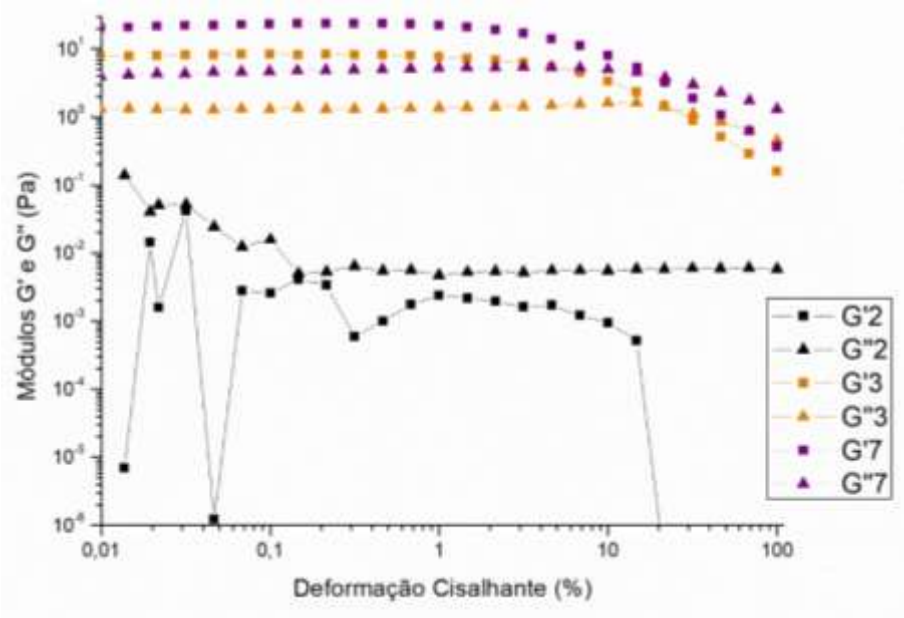
3.1.5. Viscoelastic characterization: rheology

Amplitude sweep (triplicates) and frequency sweep rheological assays were conducted using Anton Paar RheoCompass 1.31, with plots generated in Origin 7.0. Rheological behavior of sequence 2–3–7 (Figure 13) revealed that sample 2 exhibited viscous liquid dominance (G″ > G′), while samples 3 and 7 showed viscoelastic solid dominance (G′ > G″).

Figure 13 - Comparative graph of G moduli as a function of shear deformation between samples 2 - 3 - 7.



Source: From autor (2025)

Figure 14 - Comparative graph of G moduli as a function of shear deformation between samples 2 - 4 - 8.

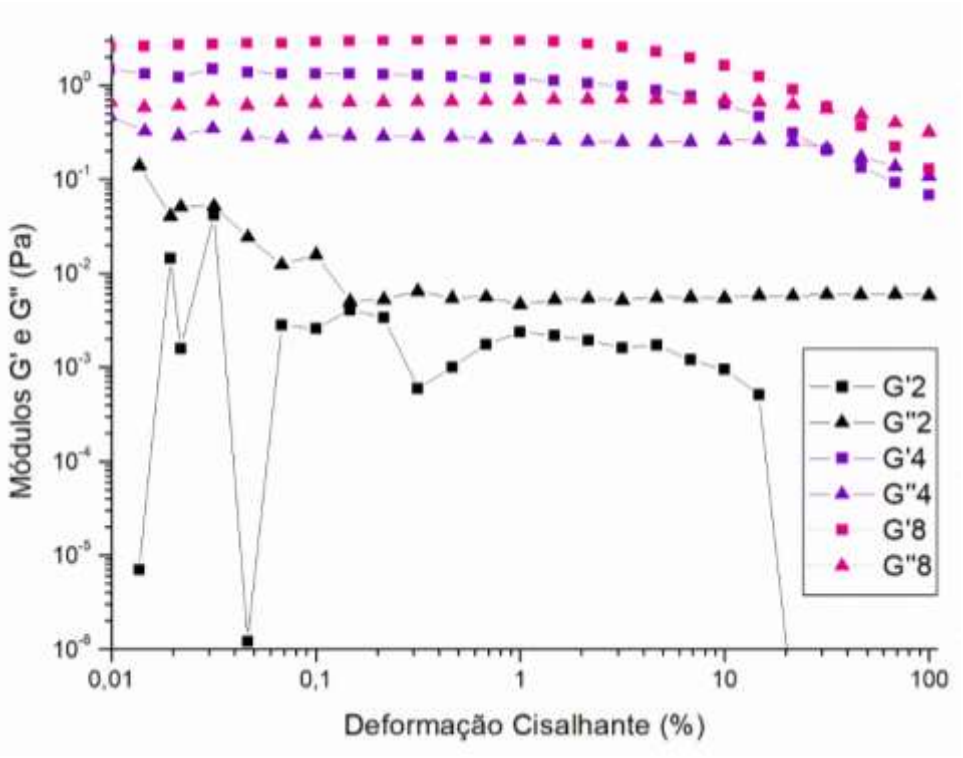


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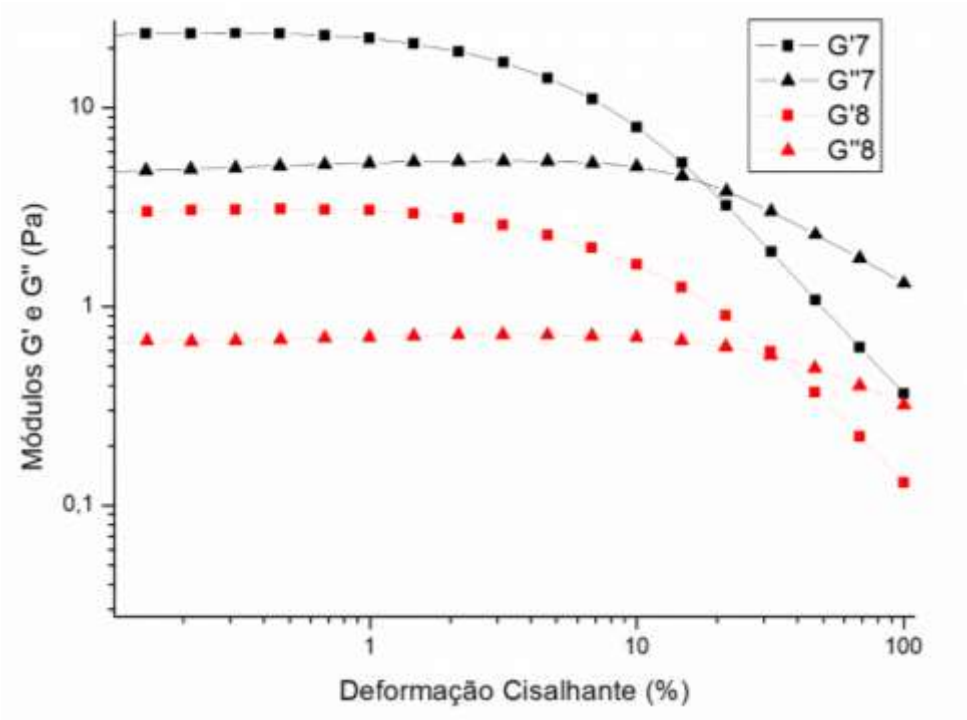
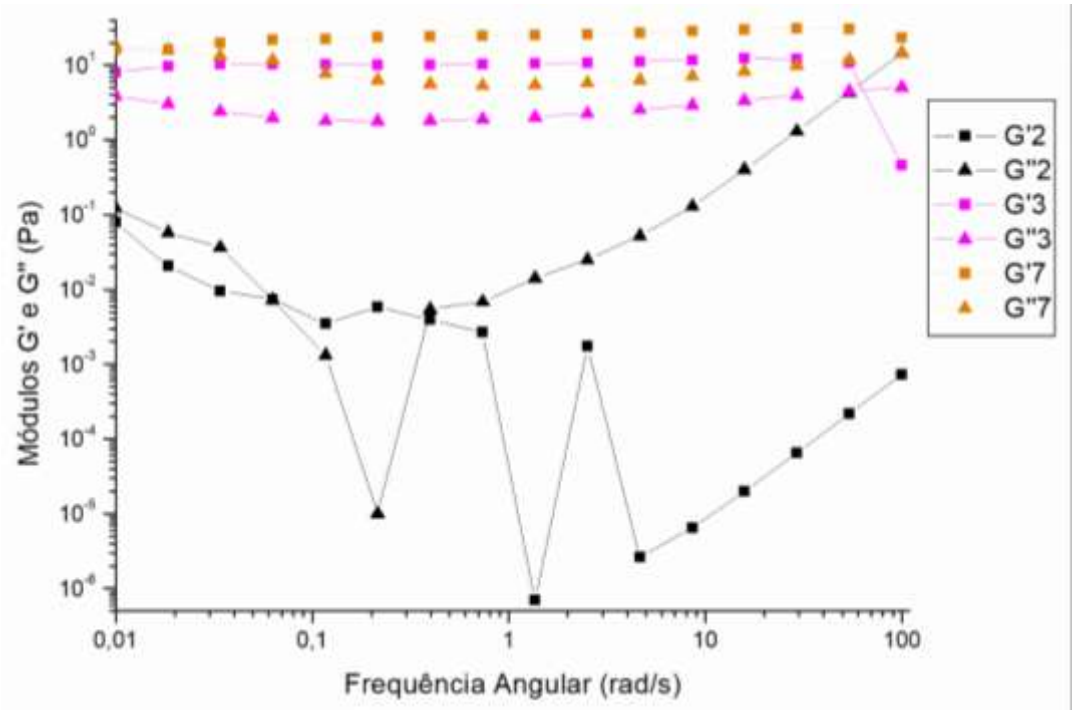
Figure 15 - Comparative graph of G moduli as a function of shear deformation between samples 7 - 8.



Source: From autor (2025)

Figure 16 - Comparative graph of G-Modules as a function of angular frequency between samples 2 - 3 - 7.



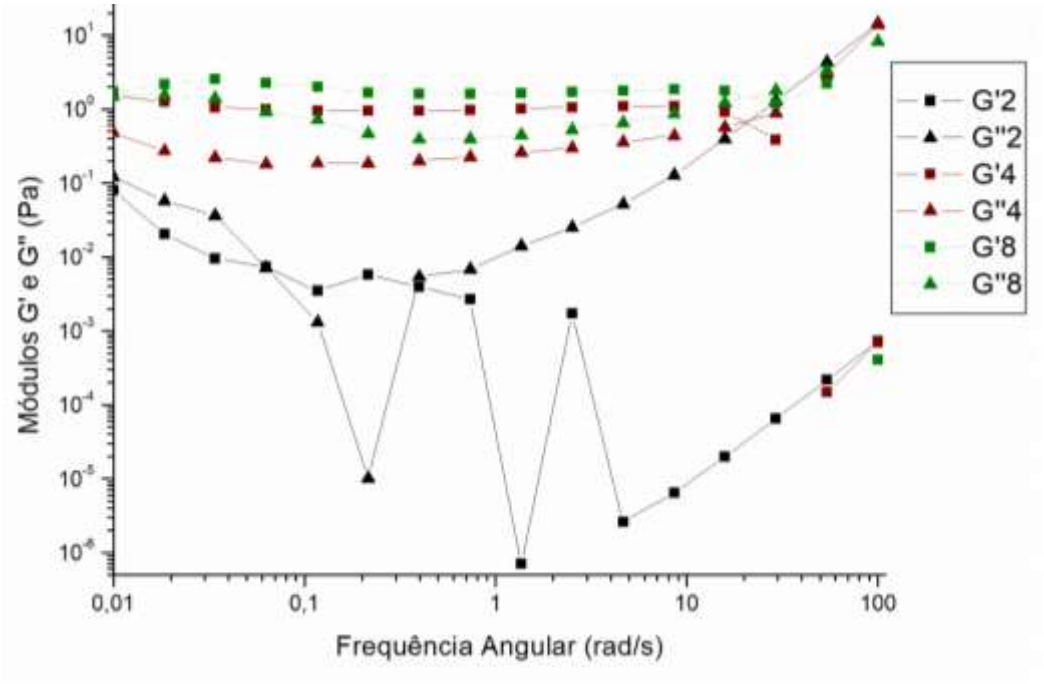
Source: From autor (2025)

Similarly, rheological behavior of sequence 2–4–8 (Figure 14) followed the same pattern: sample 2 exhibited viscous liquid dominance (G″ > G′), while samples 4 and 8 behaved as viscoelastic solids (G′ > G″). These results highlight the strong influence of CMF on emulsion structural integrity, as its presence shifts the system from viscous liquid to viscoelastic solid. Samples 3–7 and 4–8 both exhibited a linear viscoelastic region (LVER) within shear strain ranges of 0.01–21.7% and 0.01–31.67%, respectively. Beyond these points, G′ and G″ values inverted, indicating transition to a new viscous liquid structure under applied shear stress.

Comparison between samples 7 and 8 (Figure 15) revealed a one-order-of-magnitude difference (10^1) in G′ and G″, potentially associated with CMF particle concentration, as sample 7 contained more solids than sample 8.

When analyzing the rheological behavior of sequence 2–3–7 with respect to angular frequency (Figure 16), an unstable relationship between G′ and G″ is observed in sample 2. In contrast, samples 3 and 7 already display a more stable behavior, with G′ > G″ within the low-frequency range (0.01 rad/s to 1 rad/s) and maintaining stability at higher frequencies, approaching 100 rad/s.

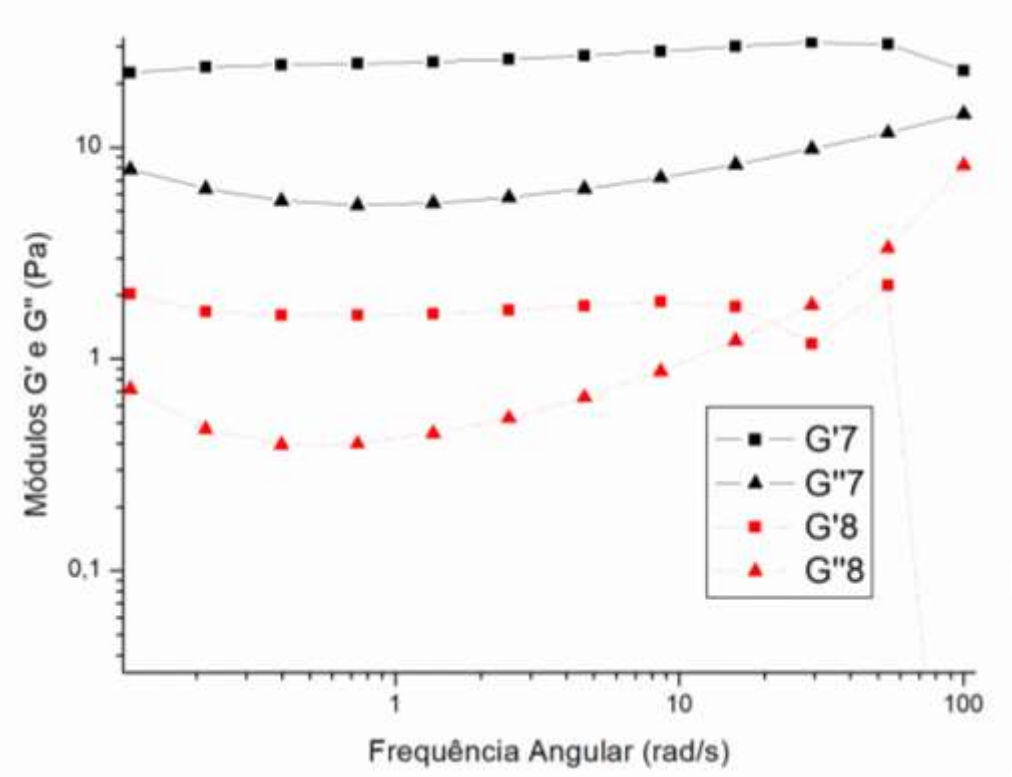
Figure 17 - Comparative graph of G-Modules as a function of angular frequency between samples 2 - 4 - 8.



Source: From autor (2025)

In sequence 2–4–8 (Figure 17), samples 4 and 8 exhibited stable behavior across frequencies of 0.01–16 rad/s, with sample 8 maintaining solid-like dominance below 16 rad/s before shifting at higher frequencies. Comparing samples 7 and 8 (Figure 18) showed not only higher G′ and G″ values for sample 8 but also a rightward shift of its phase inversion point relative to sample 7, suggesting that higher CMF solids enhance emulsion robustness, conferring greater short- and long-term stability. Rheological analyses confirm the fundamental role of CMF as a thickening and stabilizing agent in emulsified systems. Its mere presence is sufficient to alter formulation behavior, converting it from a viscous liquid to a stable viscoelastic solid at both low and high frequencies (Qiao et al., 2023; Xu et al., 2018).

Figure 18 - Comparative graph of G-Modules as a function of angular frequency between samples 7 - 8.



Source: From autor (2025)

4. CONCLUSION

Initially, an attempt was made to stabilize castor oil in an O/W system using Tween 80 and ethanol as emulsifiers, in a 1:2 ratio adapted from Nazar et al. (2009). Tests were carried out with two proportions of the T:E combination, 50% and 70%, which served as control groups. The samples exhibited visual instability on the first day after formulation, indicating that the conditions employed were not sufficient to achieve prolonged stability.

A control formulation was also prepared using only CMF and castor oil, in which the two emulsions differed only in the CMF solid content (0.45% and 0.25%). These samples also showed instability within one day after preparation, suggesting that CMF alone was not sufficient to ensure prolonged stability.

In contrast, the association of T:E (70%) with CMF dispersions (0.25% and 0.45% solid content) demonstrated effective stability throughout the seven-day observation period, indicating that CMF is capable of imparting extended apparent stability to the emulsion system.

Regarding microstructure and droplet size distribution, the presence of CMF did not present statistical differences compared to the control (sample 2), leading to the conclusion that CMF can preserve droplet dispersion in the emulsion, regardless of the proposed solid content.

The ionic demand analysis revealed an interesting relationship between CMF and the Tween 80–ethanol combination: CMF exhibited anionic charge behavior in the formulation, whereas T:E was nonionic. Although this analysis did not allow definitive conclusions about their interaction in relation to emulsion stability, the findings suggest the importance of further investigating CMF charge behavior in interactions with other components, in order to better understand the performance of its network within the system, given that it is formed by repulsive surface forces (Geng et al., 2018; Lee et al., 2020; Pinto et al., 2019).

With respect to viscoelastic behavior, the amplitude sweep showed that the presence of CMF places the emulsion within a stable linear viscoelastic region (LVER) with a solid-like viscoelastic character, while the frequency sweep indicated structural stability at both low and high frequencies. Therefore, it can be concluded that CMF contributes to structural stability in the emulsion, while variations in fiber content highlight its thickening effect.

For future work it is suggested the application of a longer stability period followed through the analysis of physical-chemical behavior in the interaction between tween 80, alcohol, CMF and oil droplets.

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APPENDIX I – ANOVA OF SAMPLES DIAMETERS DISTRIBUITION

Number of families	1
Number of comparisons per family	10
Alpha	0,05

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant ?	Summary	Adjusted P Value	
A2 vs. A3	6,304	-1,854 to 14,46	No	ns	0,2158	A-B
A2 vs. A4	10,91	2,796 to 19,02	Yes	**	0,0023	A-C
A2 vs. A7	-5,877	-12,71 to 0,9575	No	ns	0,1303	A-D
A2 vs. A8	-2,13	-8,364 to 4,103	No	ns	0,8837	A-E

A3 vs. A4	4,602	-5,094 to 14,30	No	ns	0,6931	B-C
A3 vs. A7	-12,18	-20,84 to -3,523	Yes	**	0,0012	B-D
A3 vs. A8	-8,434	-16,63 to -0,2427	Yes	*	0,0399	B-E
A4 vs. A7	-16,78	-25,40 to -8,171	Yes	****	<0,0001	C-D
A4 vs. A8	-13,04	-21,18 to -4,893	Yes	***	0,0001	C-E
A7 vs. A8	3,747	-3,127 to 10,62	No	ns	0,5695	D-E

Test details	Mean 1	Mean 2	Mean Diff,	SE of diff,	n1	n2	q	DF
A2 vs. A3	36,68	30,37	6,304	2,986	292	118	2,986	1012
A2 vs. A4	36,68	25,77	10,91	2,968	292	120	5,197	1012
A2 vs. A7	36,68	42,56	-5,877	2,501	292	203	3,323	1012
A2 vs. A8	36,68	38,81	-2,13	2,281	292	284	1,321	1012
A3 vs. A4	30,37	25,77	4,602	3,548	118	120	1,834	1012
A3 vs. A7	30,37	42,56	-12,18	3,168	118	203	5,437	1012
A3 vs. A8	30,37	38,81	-8,434	2,998	118	284	3,979	1012
A4 vs. A7	25,77	42,56	-16,78	3,152	120	203	7,531	1012
A4 vs. A8	25,77	38,81	-13,04	2,98	120	284	6,187	1012
A7 vs. A8	42,56	38,81	3,747	2,516	203	284	2,107	1012

APPENDIX II – TWO-WAY ANOVA OF IONIC DEMAND

Comparação entre os dias

Compare row means (main row effect)

Number of families	1
Number of comparisons per family	6
Alpha	0,05

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant ?	Summary	Adjusted P Value
1º vs. 2º	-10,8	-31,58 to 9,977	No	ns	0,4573
1º vs. 4º	0,333	-22,55 to 23,22	No	ns	>0,9999
1º vs. 7º	17,53	-19,92 to 54,99	No	ns	0,5423
2º vs. 4º	11,13	-18,14 to 40,41	No	ns	0,6922

2º vs. 7º	28,33	-12,41 to 69,08	No	ns	0,2267
4º vs. 7º	17,2	-22,50 to 56,90	No	ns	0,6017

Test details	Mean 1	Mean 2	Mean Diff,	SE of diff,	N1	N2	q	DF
1º vs. 2º	131,6	142,4	-10,8	7,148	15	15	2,137	14
1º vs. 4º	131,6	131,3	0,3333	7,874	15	15	0,05987	14
1º vs. 7º	131,6	114,1	17,53	12,89	15	15	1,924	14
2º vs. 4º	142,4	131,3	11,13	10,07	15	15	1,563	14
2º vs. 7º	142,4	114,1	28,33	14,02	15	15	2,859	14
4º vs. 7º	131,3	114,1	17,2	13,66	15	15	1,781	14

Comparação entre as amostras

Within each row, compare columns (simple effects within rows)

Number of families	4
Number of comparisons per family	10
Alpha	0,05

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant ?	Summary	Adjusted P Value
1º					
A2 vs. A3	-156,3	-193,1 to -119,5	Yes	**	0,0017
A2 vs. A4	-266,3	-335,0 to -197,7	Yes	**	0,0026
A2 vs. A7	-46	-151,9 to 59,88	No	ns	0,2287
A2 vs. A8	-26	-43,16 to -8,839	Yes	*	0,0189
A3 vs. A4	-110	-163,5 to -56,51	Yes	**	0,0061
A3 vs. A7	110,3	20,41 to 200,3	Yes	*	0,0308
A3 vs. A8	130,3	100,3 to 160,4	Yes	***	0,0009
A4 vs. A7	220,3	140,2 to 300,4	Yes	**	0,0019
A4 vs. A8	240,3	178,4 to 302,2	Yes	**	0,0024
A7 vs. A8	20	-80,64 to 120,6	No	ns	0,6721

2º					
A2 vs. A3	-154,7	-343,7 to 34,40	No	ns	0,0739
A2 vs. A4	-282,3	-477,8 to -86,88	Yes	*	0,0241
A2 vs. A7	-65,67	-197,1 to 65,79	No	ns	0,1881
A2 vs. A8	-36	-75,04 to 3,038	No	ns	0,0612
A3 vs. A4	-127,7	-288,3 to 32,98	No	ns	0,1022
A3 vs. A7	89	-56,02 to 234,0	No	ns	0,1923
A3 vs. A8	118,7	-56,78 to 294,1	No	ns	0,1124
A4 vs. A7	216,7	66,99 to 366,3	Yes	*	0,0155
A4 vs. A8	246,3	64,28 to 428,4	Yes	*	0,0261
A7 vs. A8	29,67	-86,65 to 146,0	No	ns	0,6042
4º					
A2 vs. A3	-136,7	-208,8 to -64,55	Yes	**	0,0057
A2 vs. A4	-247,7	-477,7 to -17,68	Yes	*	0,0425
A2 vs. A7	-47,67	-109,5 to 14,21	No	ns	0,0909
A2 vs. A8	-22,67	-81,42 to 36,09	No	ns	0,3889
A3 vs. A4	-111	-328,4 to 106,4	No	ns	0,2153
A3 vs. A7	89	3,995 to 174,0	Yes	*	0,0452
A3 vs. A8	114	32,76 to 195,2	Yes	*	0,0225
A4 vs. A7	200	-53,98 to 454,0	No	ns	0,0794
A4 vs. A8	225	-26,49 to 476,5	No	ns	0,0623
A7 vs. A8	25	-0,4790 to 50,48	No	ns	0,0531
7º					
A2 vs. A3	-129,7	-506,8 to 247,4	No	ns	0,3294
A2 vs. A4	-202,7	-319,0 to -86,36	Yes	*	0,0158
A2 vs. A7	-63,67	-207,5 to 80,18	No	ns	0,2309

A2 vs. A8	-39,33	-57,18 to -21,49	Yes	**	0,003
A3 vs. A4	-73	-401,3 to 255,3	No	ns	0,6715
A3 vs. A7	66	-247,9 to 379,9	No	ns	0,7391
A3 vs. A8	90,33	-287,4 to 468,1	No	ns	0,5275
A4 vs. A7	139	24,77 to 253,2	Yes	*	0,0262
A4 vs. A8	163,3	45,47 to 281,2	Yes	*	0,0257
A7 vs. A8	24,33	-120,9 to 169,5	No	ns	0,7456

Test details	Mean 1	Mean 2	Mean Diff,	SE of diff,	N1	N 2	q	DF
1º								
A2 vs. A3	32,67	189	-156,3	5,11	3	3	43,27	2,123
A2 vs. A4	32,67	299	-266,3	9,117	3	3	41,32	2,038
A2 vs. A7	32,67	78,67	-46	13,89	3	3	4,684	2,016
A2 vs. A8	32,67	58,67	-26	2,749	3	3	13,38	2,453
A3 vs. A4	189	299	-110	10,38	3	3	14,99	3,124
A3 vs. A7	189	78,67	110,3	14,75	3	3	10,58	2,518
A3 vs. A8	189	58,67	130,3	5,667	3	3	32,53	2,999
A4 vs. A7	299	78,67	220,3	16,57	3	3	18,81	3,448
A4 vs. A8	299	58,67	240,3	9,44	3	3	36,01	2,327
A7 vs. A8	78,67	58,67	20	14,1	3	3	2,006	2,141
2º								
A2 vs. A3	34,67	189,3	-154,7	25,29	3	3	8,649	2,051
A2 vs. A4	34,67	317	-282,3	26,1	3	3	15,3	2,048
A2 vs. A7	34,67	100,3	-65,67	18,06	3	3	5,142	2,102
A2 vs. A8	34,67	70,67	-36	6,944	3	3	7,332	2,777
A3 vs. A4	189,3	317	-127,7	36,12	3	3	4,999	3,996
A3 vs. A7	189,3	100,3	89	30,81	3	3	4,085	3,607
A3 vs. A8	189,3	70,67	118,7	25,91	3	3	6,476	2,253
A4 vs. A7	317	100,3	216,7	31,48	3	3	9,733	3,545
A4 vs. A8	317	70,67	246,3	26,7	3	3	13,05	2,238
A7 vs. A8	100,3	70,67	29,67	18,93	3	3	2,217	2,496
4º								
A2 vs. A3	40,33	177	-136,7	15,68	3	3	12,33	3,754
A2 vs. A4	40,33	288	-247,7	34,98	3	3	10,01	2,321
A2 vs. A7	40,33	88	-47,67	10,19	3	3	6,617	2,53
A2 vs. A8	40,33	63	-22,67	10,51	3	3	3,05	2,797

A3 vs. A4	177	288	-111	35,87	3	3	4,377	2,535
A3 vs. A7	177	88	89	12,91	3	3	9,749	2,318
A3 vs. A8	177	63	114	13,17	3	3	12,25	2,485
A4 vs. A7	288	88	200	33,83	3	3	8,361	2,044
A4 vs. A8	288	63	225	33,93	3	3	9,379	2,067
A7 vs. A8	88	63	25	5,598	3	3	6,316	3,827
7º								
A2 vs. A3	27	156,7	-129,7	49,43	3	3	3,71	2,015
A2 vs. A4	27	229,7	-202,7	16,29	3	3	17,59	2,14
A2 vs. A7	27	90,67	-63,67	19,69	3	3	4,572	2,095
A2 vs. A8	27	66,33	-39,33	3,972	3	3	14	3,922
A3 vs. A4	156,7	229,7	-73	51,87	3	3	1,99	2,417
A3 vs. A7	156,7	90,67	66	53,04	3	3	1,76	2,608
A3 vs. A8	156,7	66,33	90,33	49,41	3	3	2,586	2,011
A4 vs. A7	229,7	90,67	139	25,2	3	3	7,8	3,857
A4 vs. A8	229,7	66,33	163,3	16,22	3	3	14,24	2,106
A7 vs. A8	90,67	66,33	24,33	19,64	3	3	1,753	2,072