Characterization and evaluation of Cyanoflan as a novel cosmetic ingredient

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Resumo

A pele está constantemente sujeita a ameaças ambientais, como radiação, substâncias tóxicas, forças mecânicas e invasões patológicas, que podem levar a uma resposta imunológica diminuída, aceleração do envelhecimento e aumento do risco de desenvolvimento de vários problemas da pele. Assim, os produtos cosméticos desempenham um papel essencial na manutenção de uma pele saudável. Nos últimos anos, a sustentabilidade na indústria cosmética tem suscitado um interesse crescente por parte dos consumidores e por consequência dos produtores dos cosméticos. Embora o uso de ingredientes considerados mais sustentáveis possa ser um grande desafio devido à possível menor eficácia, estabilidade e limitações estéticas associadas ao seu uso, o desenvolvimento de novos ingredientes naturais, seguros e rentáveis é de extrema importância. Neste contexto, compostos derivados de cianobactérias têm-se mostrado promissores para ultrapassar estes desafios. Um dos aspetos mais relevantes das cianobactérias é a sua capacidade de produzir uma ampla gama de compostos bioativos, incluindo polissacarídeos extracelulares. Os polissacarídeos de cianobactérias possuem propriedades físico-químicas únicas que os tornam altamente adequados para várias aplicações industriais, especialmente na indústria cosmética. Posto isto, neste estudo, o objetivo principal foi caracterizar e avaliar a aplicação do heteropolissacarídeo Cyanoflan em formulações destinadas à proteção e regeneração da pele humana, com foco na indústria cosmética.

Para esse fim, a cianobactéria marinha *Crocosphaera chwakensis* CCY0110 foi crescida sob condições ambientais controladas com monitorização contínua do crescimento. Após alcançar uma quantidade de biomassa ótima, o polímero Cyanoflan foi extraído e purificado, tendo sido obtido um rendimento de 800 mg/L. Para investigar as propriedades antioxidantes do Cyanoflan, soluções com diferentes concentrações do polímero foram preparadas e submetidas a dois ensaios distintos. Ambos os ensaios revelaram uma atividade antioxidante diretamente correlacionada com a concentração de Cyanoflan (até 1% p/v).

Testes adicionais envolveram o desenvolvimento de várias formulações de Cyanoflan combinado com compostos cosméticos comumente utilizados. Estas formulações foram avaliadas quanto à sua compatibilidade a longo prazo, analisando propriedades organoléticas, estabilidade de pH e comportamento reológico. Avaliações visuais e olfativas não mostraram alterações significativas em nenhuma das formulações ao longo do tempo, e os níveis de pH permaneceram relativamente

estáveis. Os testes reológicos demonstraram que todas as soluções exibiram comportamento não-Newtoniano. Notavelmente, formulações contendo $MgSO_4$, ácido lático e o dodecil sulfato de sódio mostraram valores de viscosidade aparente ligeiramente mais elevados, indicando a necessidade de testes adicionais para uma melhor compreensão deste comportamento. Foi dada especial atenção à interação entre o Cyanoflan e outro ingrediente natural com propriedades espessantes, a goma xantana. O efeito sinérgico destes dois polímeros naturais resultou numa alta viscosidade, com valores próximos aos do polímero sintético estudado, o carbómero. Em geral, o Cyanoflan demonstrou compatibilidade com todos os compostos testados e manteve a estabilidade ao longo do tempo.

Este estudo destaca o potencial do Cyanoflan como um ingrediente benéfico em formulações cosméticas, proporcionando uma base sólida para futuras pesquisas destinadas a explorar a sua eficácia e aplicação na indústria cosmética. As propriedades promissoras demonstradas pelo Cyanoflan, tais como antioxidante, estabilizante e espessante sublinham o seu valor como um ingrediente natural, eficaz e sustentável para produtos relacionados com a proteção e regeneração da pele.

Palavras-chave: cianobactérias, Cyanoflan, cosméticos, potencial antioxidante, efeito espessante.

Abstract

Skin is constantly subjected to environmental threats, such as radiation, toxic substances, mechanical forces, and pathological invasion, which can lead to a diminished immune response, acceleration of skin aging and increase the risk of developing several skin conditions. Therefore, cosmetic products play an essential role in the maintenance of a healthy skin. In recent years, sustainability on the cosmetic industry has received growing interest from consumers and subsequently from the cosmetic producers. Although, the use of ingredients considered more sustainable can be quite challenging due to possible under performance, instability and aesthetic limitations associated with their use, so it is important to focus on research and development of novel, safe and cost-effective natural ingredients with appropriate performance. In this context, compounds derived from cyanobacteria have shown to be promising in addressing these challenges. One of the most promising aspects of cyanobacteria is their ability to produce a wide range of bioactive compounds, including extracellular polysaccharides. These cyanobacterial polysaccharides possess unique physicochemical properties that make them highly suitable for various industrial applications, particularly in the field of cosmetics. Therefore, in this study, the major aim was to characterize and assess the application of the cyanobacterial heteropolysaccharide Cyanoflan in formulations designed for the protection and regeneration of human skin, with a focus on the cosmetic industry.

For that purpose, the marine cyanobacterium *Crocosphaera chwakensis* CCY0110 was grown under controlled environmental conditions with continuous monitoring to evaluate its growth. Upon reaching an optimum amount of biomass, the polymer Cyanoflan was extracted and purified, resulting in a yield of 800 mg/L. To investigate the antioxidant properties of Cyanoflan, solutions of varying concentrations were prepared and subjected to two distinct assays. Both assays revealed a dose-dependent antioxidant activity, with increasing Cyanoflan concentrations up to 1% (w/v).

Further research involved development of various formulations of Cyanoflan combined with commonly used cosmetic compounds. These formulations were assessed for their long-term compatibility by analysing the organoleptic properties, pH stability, and rheological behaviour. Visual and olfactory assessments showed no significant changes in any of the formulations over time, and the pH levels remained relatively stable. The rheological tests demonstrated that all solutions exhibited non-Newtonian behaviour. Notably, formulations containing $MgSO_4$, lactic acid, and sodium

dodecyl sulfate showed slightly elevated apparent viscosity values at certain time points, indicating the need for further testing to better understand this behaviour. Special attention was also given to the interaction between Cyanoflan and another natural ingredient with thickening properties, xanthan gum. The synergistic effect of these two natural polymers resulted in high viscosity, with values approaching those of the synthetic polymer studied, carbomer. Overall, Cyanoflan was compatible with all tested compounds and maintained stability over time.

This study highlights the potential of Cyanoflan as a beneficial ingredient in cosmetic formulations, providing a strong foundation for future research aimed at exploring its efficacy and applications in the cosmetic industry. The promising antioxidant properties, stability of Cyanoflan and its thickening properties underscore its value as a natural, effective, and sustainable ingredient for skin protection and regeneration related products.

Keywords: cyanobacteria, Cyanoflan, cosmetics, antioxidant potential, thickening effect.

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List of Abbreviations

ABC	ATP binding cassette transporter
ABTS	[2,2´-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)]
ASNIII	Artificial Seawater
CCY	Culture Collection of Yerseke
DMAB	4-dimethylamino(benzaldehyde)
DPPH	2,2-diphenyl-1-picrylhydrazyl
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EPS	Extracellular polymeric substances
GAG	Glycosaminoglycan
HAs	Hydroxy acids
MAAs	Mycosporine-like amino acids
OD	Optical Density
OPX	Outer membrane polysaccharide export
PBP	Phycobiliproteins
PCP	Polysaccharide copolymerase
R.P.M	Revolutions per minute
ROS	Reactive oxygen species
RPS	Released polysaccharides
RT	Room temperature
SA	Scavenging activity
SC	Stratum Corneum
SCY	Scytonemin
SDS	Sodium dodecyl sulfate
TAC	Total antioxidant capacity
TM	Tropic Marine

1. Introduction

1.1. Cosmetics and cosmeceuticals

Skin, the largest organ in the human body, serves as the body's first line of defense against external environmental threats such as radiation, toxic substances, mechanical forces, and pathological invasion (Berthon et al., 2017). Furthermore, skin is constituted by the epidermis, dermis, and hypodermis, each with a distinct function. The outermost layer of the epidermis, known as the stratum corneum (SC), is made up of deceased cells. The dermis, located in the middle, primarily consists of extracellular matrix (ECM) produced by fibroblasts, which plays a crucial role in the skin's synthesis of substances like hyaluronic acid, collagen, elastin and proteoglycans, all being vital in providing structural support for cell adhesion and migration, leading to cell growth and survival. The hypodermis, the innermost layer of the skin, contains fat and connective tissue that insulates the body and protects internal organs (Xie et al., 2024). However, this natural structure of the skin can be altered by both internal and external causes, such as immunological disorders, stress, toxic substances, reactive oxygen species (ROS), or UV radiation. These disruptions to the skin can lead to inflammation, oxidative stress, imbalanced epidermal homeostasis, and a diminished immune response, accelerating skin aging and increasing the risk of developing several skin conditions, including psoriasis, skin cancer, and atopic dermatitis (Jang & Kim, 2021; Rodríguez-Luna et al., 2018).

The importance of a healthy skin drives the creation of topical products to protect against exogenous and endogenous injurious agents. Most of these products are regulated as cosmetics. A cosmetic product is defined by the European Parliament according to the Regulation (EC) No 1223/2009 as "any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity, with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours" (European Parliament, 2009). To enhance the effects of cosmetic products with pharmaceutical benefits, certain active formulations known as cosmeceuticals are incorporated. Cosmeceuticals represent the merging of cosmetics and pharmaceuticals, containing active ingredients in high concentrations. With their inherent properties for treatment and prevention, cosmeceuticals are at the forefront of skincare (Augustine et

al., 2013). They boast a higher scientific profile compared to standard cosmetics but possess a lower therapeutic value than pharmaceutical products. Applied topically like cosmetics, cosmeceuticals contain ingredients that impact the skin's biological functions, providing essential nutrients for promoting healthy skin appearance (Augustine et al., 2013). Over the past few years, the use of cosmetics and cosmeceuticals has had a significant growth, with the global cosmetics/cosmeceuticals market estimated to increase significantly by 2026 (Global Cosmetics Market Report, 2022).

A standard cosmetic product comprises around 15 to 50 ingredients, which may include water, moisturizers, emulsifiers, surfactants, thickeners, colorants, antioxidants, UV filters and fragrances (Bom et al., 2019). The components within a cosmetic formulation serve various purposes, including safety, effectiveness, sensory characteristics, and maintaining physical and chemical stability. Consumers are now seeking cosmetics that are made with less synthetic components, and that are also environmentally friendly. Synthetic chemicals in cosmetics can be detrimental and promote skin aging. Additionally, their natural counterparts can be mild, biodegradable, safe, and possess less side effects and higher biocompatibility. An interesting example in this area is biotechnology applications centered on aquatic organisms, particularly algae and cyanobacteria (Morone et al., 2019).

1.1.1. Thickeners

Cosmetics are complex products, therefore understanding their texture and flow behavior is crucial for a good formulation. Rheology is used to study the deformation and flow behavior of materials. A substance known as a rheology modifier alters the rheology of the fluid composition in which it is introduced, and this is essential to ensure that the desired flow characteristics of the cosmetic product is obtained (Calvo et al., 2020). Solids, or very elastic materials, will deform when exposed to external forces, while liquids, or extremely viscous materials, will flow. Investigating the behavior of actual materials with characteristics halfway between ideal liquid and perfect solid is another aspect of rheology (Ricarte & Shanbhag, 2024). Most fluids flow characteristics fall into one of two categories: Newtonian or non-Newtonian. The viscosity and stress behavior of Newtonian fluids are not dependent on the shear rate. In contrast, non-Newtonian fluids with shear thinning behavior, demonstrate how a solution’s viscosity behavior is influenced by the shear rate, where viscosity decreases with the increasing of the shear rate. This property, commonly observed in polymer solutions, is essential for most cosmetic products and is typically achieved through the incorporation of thickeners,

providing the cosmetic formulation with the desired consistency (Kaneda, 2017; Sangroniz et al., 2023).

Thickeners are crucial in providing cosmetic products with their desired consistency. They are divided into four categories: lipid-based, mineral-based, naturally derived, and synthetic (Lochhead, 2017). Lipid thickeners, such as cetyl alcohol and waxes, are natural and solid at room temperature, typically added to formulations after liquefaction. Mineral thickeners, such as silica and magnesium aluminum silicate, are also natural and work by absorbing water and oil to enhance viscosity. The remaining two groups primarily consist of polymers, which serve various functions beyond thickening, including stabilization, emulsification, and modification of rheology (Lochhead, 2017). Naturally derived thickeners, such as xanthan gum, gelatin, and guar gum, are polymers that absorb water and swell, increasing viscosity. They can be either natural or semi-synthetic (Alves et al., 2020). Synthetic thickeners, on the other hand, are versatile synthetic polymers providing benefits such as excellent sensory properties, cost-effectiveness, high viscosity at low concentrations, and long-term stability. Examples include carbomers, poloxamers and ammonium acryloyldimethyltaurate. Despite their advantageous properties, synthetic thickeners raise sustainability concerns due to their petrochemical origins and potential environmental impact, particularly their non-biodegradability and detrimental effects on aquatic ecosystems. Although natural polymers emerge as a more sustainable alternative due to their biodegradability, biocompatibility, and non-toxicity, they may exhibit disadvantages such as lower performance (Alves et al., 2020; Bom et al., 2019). It is therefore necessary to research more natural options with the appropriate properties, as well as studying the impact of different combinations of thickeners and/or other types of ingredients.

1.1.2. Antioxidants

Antioxidants are compounds or systems designed to interact safely with free radicals, thereby interrupting the chain reaction that could otherwise damage crucial molecules. They can use different processes to achieve this: scavenging species that initiate peroxidation, chelating metal ions to prevent their participation in reactive processes or decompose peroxides, reducing superoxide radicals to delay peroxide formation, interrupting the auto-oxidative chain reaction and/or decreasing oxygen levels in specific areas (Oroian & Escriche, 2015). Various factors such as genetic predisposition, excessive sunlight exposure, pollution, stress, and substance abuse like alcohol and smoking, can trigger oxidative stress in tissues by generating high levels of

reactive oxygen species (ROS). These ROS, such as singlet oxygen ($^1O_2$), superoxide radical (O_2^-), hydroxyl radical (OH●), and hydrogen peroxide (H_2O_2), are constantly produced in cells during metabolic processes. Oxidative stress contributes to premature aging, the formation of wrinkles, inflammation, and potential malignant changes in cells of the skin (Oresajo et al., 2012). To prevent the harmful effects of free radicals, the skin relies on antioxidants, which either enzymatically or non-enzymatically neutralize the ROS within cells. While oxidation reactions are vital for life an excess of ROS can overwhelm the body's antioxidant defense system, leading to the oxidation of proteins, lipids, or DNA, which is why antioxidants are essential in a cosmetic formulation, as they have the capacity to prevent the occurrence and reduce the severity of skin damage and aging (Masaki, 2010; Oresajo et al., 2012). Furthermore, cosmetic items typically contain fats, oils, and lipid-based compositions, which are prone to oxidative breakdown, thereby shortening their shelf life. Incorporating antioxidants during production can effectively safeguard cosmetic products from oxidative degradation instigated by free radicals (Costa & Santos, 2017).

Antioxidants can be classified into two primary groups: natural and synthetic. Since synthetic antioxidants are more affordable and stable during the production process, they are employed more frequently than natural ones. Examples of these are butylated hydroxytoluene and butylated hydroxyanisole. But there is evidence that these substances can lead to endocrine disruption, allergies, and possibly cancer (Augustyniak et al., 2010; Okereke et al., 2015). A diverse array of natural antioxidants, including substances like vitamins, polyphenols, and carotenoids are already being used in the cometic industry. Although synthetic antioxidants currently dominate the market, there has been a growing demand for natural antioxidants in recent years. This shift can be attributed to an increasing consumer preference for organic and natural products, which are perceived to contain fewer additives and potentially fewer side effects compared to synthetic antioxidants (Costa & Santos, 2017).

1.1.3. Anti-aging effect

The term "aging" refers to the gradual deterioration of cells due to genetic, endogenous, or environmental factors, resulting in a loss of function and ultimately the death of the cells. There are two types of skin aging: extrinsic and intrinsic. Extrinsic aging is caused by external factors such as UV radiation exposure, air pollution, smoking and weather conditions such as wind and cold. Intrinsic aging is brought on by internal causes including hormone imbalances, genetic alterations, and/or vitamin shortages,

causing the skin to deteriorate in terms of increasing transparency, quality, and elasticity loss due to more prominent vascularity (Rodrigues et al., 2018). Skin aging is closely related to the production of ROS and matrix metalloproteinases (Zhao et al., 2022). In the dermis, the fibroblasts are responsible for the production of the extracellular matrix (ECM), which is mostly based on collagen and elastin, providing firmness and elasticity to the skin. With aging, the skin becomes drier, and pores and wrinkles form due to elevated levels of collagenase, a component of matrix metalloproteinase, resulting in the breakdown of collagen in the dermis and the formation of elastic fibers (Loo et al., 2023). Exposure to UV light is the primary cause of skin aging; in fact, extrinsic aging is often known as photoaging that is identified by modifications in derma connective tissue such as wrinkles, pigmented lesions, and patchy hypo pigmentations (Puizina-Ivić, 2008).

Skin moisture, UV protection, stimulation of fibroblast proliferation, and an increase in antioxidant potential are all mechanisms that contribute to slowing down skin aging (Morone et al., 2019). However, the most effective way to prevent skin aging is by maintaining skin hydration, since proper moisturization helps to preserve the skin's appearance and elasticity, while also enhancing its defense against harmful environmental factors. The skin's moisture level can be affected by how frequently it is washed with surfactant products and its ability to resist irritants. Nevertheless, there are distinct anti-aging products that act mainly by stimulating collagen and glycosaminoglycan (GAG) synthesis by fibroblasts in the epidermis, which increases the firmness and flexibility of the skin's outer layer (Alves et al., 2020). Hydroxy acids (HAs) are often used in skincare products for their moisturizing benefits, supporting the skin in these ways (Yarkent et al., 2020).

1.2. Cyanobacteria

Cyanobacteria are an ancient and diverse group of Gram-negative, photoautotrophic prokaryotes able to thrive in almost any type of habitat on the planet, with currently approximately 2000 species spread across 150 genera (Vincent, 2009; Whitton & Potts, 2012). Due to their long evolutionary history, cyanobacteria have been successfully colonizing modern habitats, which include soils, fresh and salt water, and even extreme environments (Sinha & Häder, 2008; Yoshiyuki et al., 2005). They were able to modify their lifestyles in response to the requirements of their metabolism, serving a vital role in the biogeochemical cycles of carbon, oxygen, and nitrogen (DeRuyter & Fromme, 2008). Furthermore, cyanobacteria can be unicellular, form multicellular

filaments, grow in colonies, or even in symbiosis with a variety of eukaryotic hosts (D'Agostino, 2023; Mota et al., 2022).

Through oxygenic photosynthesis, cyanobacteria can be employed as chemical factories that use sunlight as their only energy source. These microorganisms perform an autotrophic metabolism based on CO_2 fixation by using light to oxidize water and supply electrons. Some cyanobacteria can shift to anoxygenic photosynthesis in the absence of oxygen by utilizing sulfide as an electron donor (Abed & Köster, 2005; Jaiswal et al., 2022; Ma et al., 2022). Certain cyanobacteria can transform atmospheric N_2 into biologically accessible ammonia, allowing them to thrive even in nitrogen-deficient conditions. However, it is believed that some members have lost this capacity during evolution (Latysheva et al., 2012). Moreover, cyanobacteria have modest culture requirements with regard to space and nutrients, decreasing its production costs (Singh et al., 2017). Nevertheless, the commercial viability of cyanobacteria hinges on their cultivation, ranging from open ponds to sophisticated photobioreactors, which allow precise control over growth conditions and contamination prevention (Takenaka & Yamaguchi, 2014). Cyanobacteria stand out as rich reservoirs of essential nutrients, including proteins, fatty acids, vitamins, and minerals. The versatile nature of these microorganisms extends beyond their nutritional value, as they emerge as prolific sources of added-value compounds/products across biotechnological, biomedical, and industrial domains (Subashchandrabose et al., 2013).

1.2.1. Cyanobacterial extracellular polymeric substances (EPS)

A polymer consists in very large molecules composed by multiple structural units, typically connected by covalent chemical bonds. Natural polymers, commonly referred to as biopolymers, are generated by living organisms (Mitura et al., 2020). Cyanobacteria, as well as various microorganisms, possess the ability to produce extracellular polymeric substances (EPS). These EPS may persist on the cell surface in the form of sheaths (typically, a thin and compact layer that loosely envelops individual cells or clusters of cells), capsules (a thick and structurally cohesive layer closely associated with the cell surface), and/or slimes (a mucilaginous substance distributed around the cells without mirroring their specific shapes), or they may be secreted into the surrounding environment as released polysaccharides (RPS) (Mota et al., 2022; Rossi & De Philippis, 2016).

Depending on their complexity and structure, EPS can play several physiological roles, such as cell protection, since they form a physicochemical barrier around the cell,

protecting it from biotic and abiotic stress as a direct response to natural environmental factors (Flemming, 2016). Additionally, EPS can aid in the sequestration of nutrients, including minerals and critical metal cations, especially in habitats like the sea where resources are scarce. Furthermore, under unbalanced carbon/nitrogen metabolic conditions, EPS may become a sink for excess carbon (Bhunia et al., 2018). The presence of cyanobacterial EPS can also increase resistance against desiccation due to their high capacity of water retention. Moreover, they may aid in the aggregation of cells and their adherence to solid particles and surfaces, resulting in the creation of biofilms or biological soil crusts (Tiwari et al., 2019). EPS can influence cell adherence to surfaces by forming a cohesive network of three-dimensional polymers that facilitates communication between cells and extracellular enzyme retention, accumulation and stabilization (Flemming, 2016). Overall, it appears that the roles played by these polymers vary depending on the strain, the organism's natural environment or the culture medium in which it is grown (Mota et al., 2022).

Remarkably, cyanobacterial EPS exhibit distinct characteristics when compared to those synthesized by other microorganisms: (i) since their composition typically have 6–10 different monomers, amplifying the potential structural conformations of the polymer (Mota et al., 2013); (ii) can possess a strong anionic nature resulting from the presence of uronic acids, contributing to its rheological properties and water retention capacity; (iii) can have sulfate groups on its composition, which also imparts negative charges to EPS and can contributes for diverse biological activities, including anti-viral, anti-tumor, anti-thrombotic, anti-coagulant, anti-inflammatory, and anti-oxidant effects; and (iv) high hydrophobicity conferred by peptides, deoxysugars, and ester-linked acetyl groups (Di Martino, 2018; Tiwari et al., 2020). Collectively, these attributes make cyanobacterial EPS highly appealing for distinct applications, namely as adhesives, gelling agents, flocculants, and thickening agents in the food and beverage, medical and cosmetic industries (Mota et al., 2022).

The synthesis of cyanobacterial EPS normally occurs in three main steps. First, in the cytoplasm, the nucleotide sugar precursors are synthesized. Second, glycosyltransferases assemble repeated units of oligosaccharides and bind them to acceptor molecules. Repeating units are eventually polymerized and exported to the cell surface (Mota et al., 2022). One of three pathways is proposed to be involved in the last steps of bacterial EPS assembly and export, namely Wzy-, ABC transporter-, and Synthase-dependent pathways (Schmid, 2018). In the Wzy-dependent pathway, lipid-linked oligosaccharide units are flipped across the plasma membrane to the periplasm

by Wzx, where Wzy continues the polymerization. The polysaccharide copolymerase (PCP) Wzc and the outer membrane polysaccharide export protein (OPX) Wza are then involved in the export to the outer membrane (Mota et al., 2022). The ABC transporter-dependent pathway is linked to capsular polysaccharides (CPS), and the polymerization occurs at the cytoplasmic face of the plasma membrane. The ABC-transporter complex (KpsTM/KpsT) translocates the polymer across the plasma membrane and then the transmembrane complex KpsE/KpsD (PCP/OPX proteins) exports it. In the Synthase-dependent pathway, a polysaccharide synthase (e.g. Alg8 or BcsA) performs the polymerization and translocation of the polysaccharides from the cytoplasm to the periplasm. Then, the export is carried out by two independent proteins (e.g. AlgK and AlgE) or one that combines both functions (e.g. BcsC) (Mota et al., 2022). From what is none so far, it is likely that cyanobacteria display a more complex scenario than that observed for other bacteria (Pereira et al., 2015).

1.2.2. Cyanobacterial EPS in cosmetics

As the use of polysaccharides extends, their cosmetic properties, including thickening, anti-aging effects and moisturizing have garnered increasing interest (Erginer et al., 2023). The intricate long-chain structure of polysaccharides often forms the basis for these diverse functions. For instance, polar groups can effectively bind water molecules via hydrogen bonding, forming network structures that provide moisturizing benefits (Shao et al., 2015). Polysaccharides may also exhibit tyrosinase inhibition, free radical scavenging, and other activities, contributing to antioxidant effects (Deng et al., 2023). Moreover, polysaccharides can possess skin-repairing properties like cosmeceuticals, suggesting their potential as active ingredients in restorative skincare products (Morganti et al., 2019). In this context, cyanobacterial EPS became especially interesting.

Cyanobacteria, which are naturally exposed to oxidative stress, have developed effective defense mechanisms against ROS and free radicals. Polysaccharides from *Arthrospira platensis* (commonly known as Spirulina), for instance, possess antioxidant properties and contain significant antioxidants such as mycosporine-like amino acids (MAAs), scytonemin (SCY), vitamins (A, C, D, and E), carotenoids (beta-carotene and lycopene), enzymes (superoxide dismutase, glutathione peroxidase, and catalase), and phycobiliproteins (PBP) (Raposo et al., 2015; Wu et al., 2012). In the same context, it has been reported that the synthesis of SCY in *Rivularia* sp. HKAR-4 increases during UV radiation, indicating that this cyanobacterium can protect itself from UV-radiation

showing potential in photoprotection by minimizing the cellular damage from UV-induced ROS and thymine dimer formation (Rastogi et al., 2013). Cyanobacterial EPS are also capable of scavenging various free radicals like DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS ([2,2´-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)]), OH● and O_2^-, possibly due to their abundance of reducing hydroxyl groups. For instance, it was demonstrated that the polysaccharide from *Nostoc commune* could have scavenging rate of 65% for O^{2-} and 51% for OH● *in vitro*, showcasing its potent antioxidant activity (Li et al., 2011). In addition to exhibiting antioxidant properties, these EPS demonstrate significant abilities in absorbing and retaining moisture. This characteristic makes them highly promising for utilization as humectants in moisturizers, eliminating the necessity for undesirable occlusive agents (Li et al., 2011).

Cyanobacterial polysaccharides have also shown great rheological properties, which can be of highly importance for use in the cosmetic industry, especially as emulsifying and thickening agents. Particularly, EPS produced by different filamentous cyanobacteria, such as *Nostoc flagelliforme* (Han et al., 2014), *Anabaena* sp. ATCC 33047 (Moreno et al., 2000) and *Lyngbya stagnina* (Jindal et al., 2013), exhibit shear-thinning behavior, which is essential for achieving desirable properties in cosmetic formulations for topical application. In a more recent study, the polysaccharides produced by *Anabaena* sp. CCC 745 showed good values of viscosity, making this polysaccharide a possible prospective to be used as a thickener agent (Tiwari et al., 2019). Another example is the polymer Sacran, extracted from *Aphanothece sacrum*, which, especially when NaCl was added, showed increased viscosity due to the disruption of the electrostatic interactions, causing the polymer chains to become more linear and improving its capacity as a thickening agent (Okeyoshi et al., 2021).

1.2.3. The cyanobacterial polymer Cyanoflan

Crocosphera chwakensis CCY0110 is a marine unicellular cyanobacterium and a prolific producer of EPS, being the majority (>75%) released to the extracellular medium (RPS), becoming easier to isolate and purify (Mota et al., 2013). Extensive research has been conducted to understand the culture conditions influencing RPS production. Light has been identified as the crucial parameter to enhance its yield. It should be highlighted, nonetheless, that prolonged exposure to intense light can interfere with cyanobacteria's circadian cycles and lead to photoinhibition (Mota et al., 2013). The extracellular polymer produced by this cyanobacterial strain was previously named Cyanoflan (Mota et al., 2020). This biopolymer is mainly composed by carbohydrates (71% w/w dry weight),

including neutral sugars (57%) and uronic acid (14%), with the remaining being sulfated residues (11%), protein (4%), and inorganic compounds (Mota et al., 2020). In terms of monosaccharidic composition, Cyanoflan is composed by Mannose (20 mol%), Glucose (20 mol%), uronic acids (18 mol%), Galactose (10 mol%), Xylose (9 mol%), Rhamnose (9 mol%), Fucose (8 mol%), and Arabinose (6 mol%). Cyanoflan's glycosidic bond composition may be described as being highly branched, composed of several distinct sugar residues, and heavily sulfated. Moreover, Cyanoflan exhibits exceptional thermostability and high molecular mass fractions (>2 MDa) (Mota et al., 2020).

Cyanoflan has demonstrated promising capabilities across multiple domains. As an anti-adhesive coating, with broad-spectrum activity, effectively prevented the adhesion of various etiological agents and exhibited blood-compatibility and non-cytotoxicity, positioning it as a promising solution for preventing infections associated with medical devices (Costa et al., 2019). In another study, Cyanoflan demonstrated remarkable biocompatibility and facilitated the migration and proliferation of endothelial cells and fibroblasts, establishing an optimal microenvironment conducive to cell adhesion, proliferation, and migration, demonstrating Cyanoflan's potential for safe utilization as a skin dressing in biomedical settings (Costa et al., 2021). Cyanoflan has also shown promise as a drug delivery system, due to its ability to form a gel-like structure when assembled with proteins, and with the consequent controlled release of positively charged macromolecules (Leite et al., 2017). From an environmental point of view, Cyanoflan also holds promise for effective application in bioremediation, serving as a biosorbent to remove heavy metals commonly found in polluted water (Mota et al., 2016). Moreover, Cyanoflan demonstrated high apparent viscosity, non-Newtonian fluid behavior, and significant emulsifying activity akin to commercial biopolymer xanthan gum. These properties alongside with a good stability, position Cyanoflan as a versatile and a very appealing polymer for various industrial applications, particularly as an emulsifying and thickening agent in the cosmetic sector (Mota et al., 2020).

2. Aims

The main aim of this study was to further characterize and evaluate the use of the cyanobacterial polymer Cyanoflan in formulations for the protection and regeneration of human skin, targeting the cosmetic industry. To this end, the following specific objectives were established:

1. Cyanobacterial growth and isolation of Cyanoflan;
2. Evaluation of the antioxidant potential of Cyanoflan;
3. Development of formulations with known cosmetic ingredients;
4. Characterization and evaluation of the compatibility, stability and viscosity of the formulations.

3. Materials and Methods

3.1. Cyanobacterial strain and culture conditions

The cultures of *Crocosphaera chwakensis* CCY0110 (Culture Collection of Yerseke, The Netherlands; now available at Culture Collection of Algae and Protozoa – CCAP 1435/2) were grown in 2 L bioreactors with 1 L of medium, and afterwards in 5 L bioreactors (DWK Life Sciences, Germany) with 2.5 L of TM medium (Atkinson et al., 1997): modified ASNIII medium (Rippka et al., 1979), by using Tropic Marin® Classic Sea Salts (25 g L^{-1}) to replace NaCl (25 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (3.5 g L^{-1}) and $MgCl_2 \cdot 6H_2O$ (2 g L^{-1}). Cultures were maintained at 20 ºC, under a photoperiod of 16 h light (80 μE m^{-2} s^{-1})/8 h dark, with a constant aeration (1 L min^{-1}) and magnetic stirring (150 r.p.m).

3.2. Light microscopy

Cells were observed using an Olympus BX43 light microscope (Olympus, Hamburg, Germany) and stained with Alcian Blue (0.5% (w/v) in 50% ethanol) for the visualization of acid carboxylated and sulfated polysaccharides (Thornton et al., 2007).

3.3. Growth assessment

Growth measurements were performed by monitoring the optical density at 730 nm (OD_{730nm}) using a DR3900 spectrophotometer (Hach Lange, Vienna, Austria) (Anderson & McIntosh, 1991), and the content of chlorophyll *a*, which was extracted using 90% (v/v) methanol and determined spectrophotometrically as described by (Meeks & Castenholz, 1971).

3.4. Polymer isolation

The Cyanoflan was isolated according to (Costa et al., 2019). Briefly, the *Crocosphaera chwakensis* CCY0110 cultures were dialyzed against a minimum of 10 volumes of deionized water with 12-14 kDa of molecular weight cut-off dialysis membranes (Spectrum, Laguna Hills, CA, U.S.A.) for 48 h with continuous agitation. Then, the cultures were centrifuged at 15 000 xg at 10 ºC for 20 min twice, the supernatant was precipitated with 2 volumes of 96% ethanol and incubated overnight. The polymer was collected and, after adding Milli-Q water, the precipitation was repeated. After collection and freezing, the polymer was lyophilized for 48 h. Finally, the

dried polymer was grounded (Mill A10 basic, IKA, Staufen, Germany) and stored at room temperature (RT) for further use.

3.5. Antioxidant Assays

3.5.1. Cyanoflan Sample Preparation

Three different dilutions of Cyanoflan were prepared, 0.25%, 0.50% and 1.00% (w/v) in Milli-Q water. The solutions were incubated in a thermomixer at 1000 r.p.m at 50 ºC overnight. Afterwards, the solutions were autoclaved and stored at 4 ºC until further use.

3.5.2. Total Antioxidant Capacity

The total antioxidant capacity (TAC) of Cyanoflan was determined using the antioxidant assay kit (MAK334, Sigma-Aldrich, St. Louis, MO, USA) following the manufacturer's recommendations. Briefly, Cu^{2+} is reduced by an antioxidant to Cu^{+}, forming a colored complex with a dye reagent. The color intensity will be proportional to the antioxidative capacity of the sample, which is expressed in µM Trolox equivalents. The absorbance at 570nm was measured in an Infinite 200Pro Microplate Reader (Tecan, Switzerland). At least three independent tests were performed. The TAC of the samples was calculated as follows:

$$\mathrm{TAC}\ (\mu\mathrm{M}) = \frac{\mathrm{A_s} - \mathrm{A_b}}{\mathrm{Slope}\ (\mu\mathrm{M}^{-1})}$$

Where A_s is the absorbance of the sample and the A_b the absorbance of the blank.

3.5.3. ABTS Scavenging Activity

The ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] scavenging activity of Cyanoflan was measured according to (Hlima et al., 2021), with some modifications. This assay measures the relative ability of an antioxidant to scavenge $ABTS^{+}$ generated in the aqueous phase. 2.45 mM potassium persulphate was added to 7 mM ABTS and incubated in the dark at RT for 12-18h to obtain a stable oxidative state. Afterwards, the solution was diluted with ethanol until an absorbance of 0.7 ± 0.2 units at 734 nm. To test Cyanoflan antioxidant activity, 100 µL of each concentration were added to 200 µL of $ABTS^{+}$ in a 96-well plate and mixed in a thermomixer at 20 ºC for 30 s at 600 r.p.m. After 6 min of incubation at 20 ºC in the dark, the absorbance was

measured in an Infinite 200Pro Microplate Reader (Tecan, Switzerland). An aqueous solution of 0.25% (w/v) ascorbic acid was used as positive control and 96% ethanol was used as negative control. At least three independent assays were performed in triplicate. The results were expressed as the percentage of scavenging activity (SA) of $ABTS^{+}$ and as Trolox equivalents (μM) according to the formulas:

$$\text{SA}\ (\%) = \frac{\text{A}_{\text{nc}} - (\text{A}_{\text{s}} - A_b)}{\text{A}_{\text{nc}}} \times 100 \qquad \text{Trolox equivalent}\ (\mu\text{M}) = \frac{\text{A}_{\text{s}} - \text{A}_{\text{b}} - b}{\text{Slope}\ (\mu\text{M}^{-1})}$$

Where A_{nc} is the absorbance of the negative control, A_s is the absorbance of the samples and A_b the absorbance of the blanks.

3.6. Compatibility Trials

The compatibility trials were conducted by adding several common cosmetic ingredients to 0.5% and 1% (w/v) Cyanoflan in Milli-Q water (Table 1). The samples were prepared by homogenizing the Cyanoflan and the different ingredients in Milli-Q water with a homogenizer (Ultra-Turrax 25 Homogenizer, IKA) at 25 ºC and 8000 r.p.m. Organoleptic analysis was carried out to verify the following parameters: color, odor, and aspect. The pH was measured using a Mettler Toledo S220 pH meter with a combined glass electrode (Mettler-Toledo GmbH, Vienna, Austria). The assays were conducted at three time points: immediately after preparation (T_0), after 24 h stored at RT (T_{24}), and after an incubation of 6 days at 40 ºC (T_{7d}). All tests were performed in triplicate.

Table 1. Different cosmetic ingredients added to 1.0% (w/v) Cyanoflan aqueous solution.

	Ingredient	% w/v
Salt	Magnesium sulfate ($MgSO_4$)	2.0
Chelating Agent	Disodium salt EDTA	0.1
Hydrotrope	Glycerin	5.0
Alcohol	Ethanol 96%	5.0*
pH Modifier	Lactic acid (pH 3) 1 M Sodium hydroxide (NaOH) (pH 9)	10.0* To 100*
Surfactant	Sodium lauryl sulfate (SDS) Coco-glucoside	0.5 0.5*
Pigment	Zinc oxide	5.0
Thickener	Xanthan gum Carbomer 940 (pH 6)	1.0 0.5

* Indicates values in % v/v

3.6.1 Rheological Measurements

Rheology measurements of the samples described above were performed by using a modular compact rheometer equipped with a cone-plate system (CP50-1 SN65976) with a diameter of 50 mm and 0.101 mm parallel plate gap (Anton Paar MCR 302e, Graz, Austria). The measurements were performed by varying the shear rates from 0.5 to 1000 s^{-1} at 25 ºC (Viscotherm VT2). The assays were conducted at three time points as described above. All tests were performed in triplicate.

3.7. Statistical Analysis

Statistical analysis was performed with GraphPad Prism software (v.9.0.1, GraphPad software Inc. CA). Data was submitted to one-way ANOVA followed by Bonferroni's multiples comparisons test. Data is expressed as mean ± standard deviation (SD) of three independent experiments. Differences were considered significant for $p \leq 0.05$.

4. Results and Discussion

4.1. Cyanoflan production

The marine unicellular cyanobacterium *Crocosphaera chwakensis* CCY0110 was maintained and grown in a 2 L bioreactor (Fig. 1 A) and then scale up to a 5 L bioreactor. The culture was observed by optical microscopy with Alcian Blue, a specific staining for acidic polysaccharides (Fig. 1) (Thornton et al., 2007). Periodic measurements of optical density (OD_{730nm}) and chlorophyll *a* were carried out for a period of approximately 1 month until the culture reached an $OD_{730nm} \approx 2$ and 12 mg chl *a*/L, and then the polymer was isolated. At the conclusion of this process, the dried polymer was milled, resulting in a yield of approximately 2.0 g and corresponding to a concentration of about 800 mg of released polysaccharides (RPS) per L of culture. For the subsequent tests, aqueous solutions with different concentrations of Cyanoflan were formulated.

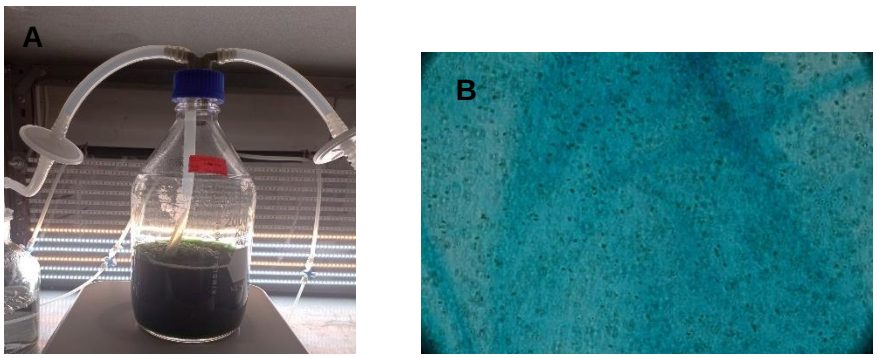

Figure 1. (A) Culture of *Crocosphaera chwakensis* CCY0110. (B) *Crocosphaera chwakensis* CCY0110 stained with Alcian Blue, highlighting the abundance and homogeneous distribution of Cyanoflan. Magnification 400x.

4.2. Assessment of Cyanoflan antioxidant capacity

4.2.1. Total Antioxidant Capacity

The Trolox equivalent of the total antioxidant capacity (TAC) of different aqueous solution of Cyanoflan (0.25%, 0.50% and 1.0%, w/v) was determined, and the results are shown in Fig. 2. The TAC is determined based on Cyanoflan capability to reduce Cu^{2+} to Cu^{+}. The occurring Cu^{+} forms a colored complex in proportion to oxidation inhibition, thus the color intensity is proportional to the TAC of Cyanoflan. The results showed that Cyanoflan's TAC is dose-dependent. At the highest concentration (1% w/v) Cyanoflan exhibited a TAC of 128.8 ± 24.9 µM Trolox equivalent, whereas at the lowest concentration (0.25% w/v) the value was 20.8 µM ± 2.6 µM Trolox equivalent. Different

methods can be applied to determine the TAC of a specific sample, making a direct comparison between results challenging. A extracellular polysaccharide from the marine protist *Thraustochytrium striatum* mainly composed by glucose and arabinose showed a TAC value between 160 and 200 µM Trolox equivalent (Xiao et al., 2018). In another study, methyl cellulose, a known biopolymer used as a thickener, was mixed with okra mucilage and presented a TAC of 190 µM Trolox equivalent (Coban et al., 2024). Different types of samples, for cosmetic applications, have also been reported with antioxidant capacities. For example, extracts from the plant *Sanguisorba minor* Scop. revealed a range of TAC values, with the lowest recorded at 1.23 and the highest at 3.26 µmol Trolox equivalent/mL (Tocai et al., 2024). Although Cyanoflan does not seem to be among the polymers with highest TAC values, it must be taken in consideration that the measurements were conducted in a relatively low concentration of Cyanoflan, approximating the realistic levels used in cosmetic formulations. Therefore, Cyanoflan antioxidant activity should be seen as part of a combination of several properties that make this biopolymer a promising ingredient for cosmetic formulations.

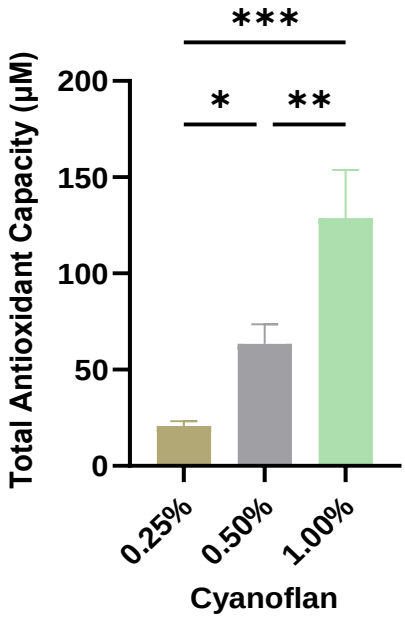

Figure 2. Total antioxidant capacity (TAC) of Cyanoflan at 0.25%, 0.50% and 1.00% (w/v) in Milli-Q water. Results are represented by µM Trolox equivalent and expressed as mean ± Standard Deviation (SD) of three independent experiments. Comparisons by one-way ANOVA followed by Bonferroni's multiples comparisons test. Statistical differences are indicated with * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

4.2.2. ABTS Scavenging Activity

To further determined Cyanoflan's antioxidant capacity, the 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) scavenging capacity assay was conducted. For a better comparison with the literature, the results are presented as percentage of scavenging activity and as µM Trolox equivalents (Fig. 3 A1 and A2, respectively). This assay demonstrated that Cyanoflan is also dose-dependent regarding the ABTS scavenging activity, with values of 27%, 52% and 63% obtained for Cyanoflan concentrations of 0.25%, 0.50% and 1.00%, respectively. The results obtained are similar to those reported in other studies. For instance, different types of flavonoids were isolated from aerial parts of *Scleranthus perennis* L. and the scavenging activity was evaluated with final values ranging from 67.94 to 765.16 µM Trolox equivalents (Jakimiuk et al., 2022). It is also possible to compare the results obtained with other marine-derived polysaccharides, mainly the polysaccharides extracted from *Porphyridium cruentum*, a unicellular red microalga (Casas-Arrojo et al., 2021). The maximum antioxidant activity detected for this polysaccharide was 2.23 ± 0.30 µmol Trolox equivalent/g at a concentration of 200 µg/mL. Polysaccharides isolated from another red microalga, *Rhodosorus* sp. SCSIO-45730, were also evaluated for its antioxidant potential (Wang et al., 2022). Similar to the results obtained for Cyanoflan, an increasing of ABTS scavenging activity was observed with the increasing concentration of the polysaccharide, reaching 64.42% of ABTS scavenging activity at a concentration of 2 mg/mL.

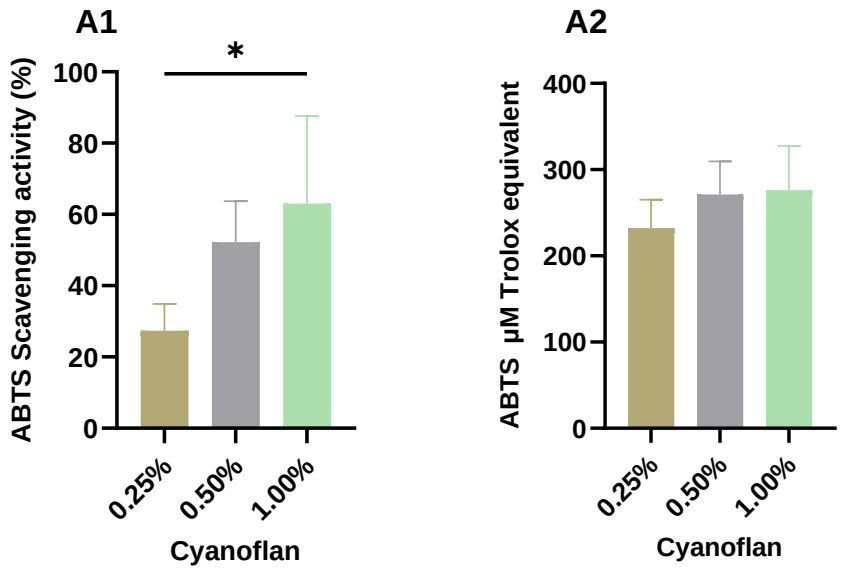

Figure 3. ABTS Scavenging Activity (SA) of Cyanoflan at 0.25%, 0.50% and 1.00% (w/v) in Milli-Q water. Results are represented by percentage (%) (A1) and µM Trolox equivalent (A2), and both expressed as mean ± Standard Deviation (SD) of four independent experiments. Comparisons by one-way ANOVA followed by Bonferroni's multiples comparisons test. Statistical differences are indicated with * $p < 0.05$.

4.3. Development and testing of cosmetic formulations

Typically, a cosmetic ingredient should have the following features: (i) organoleptic characteristics (aspect, color and odor) with low impact on the characteristics of the final formulation; (ii) easy to handle and incorporate into the formulation; (iii) stable and compatible with the most common ingredients and pH range; (iv) good tolerance to possible destabilizing ingredients, such as electrolytes, alcohol, acids, surfactants, UV filters, and pigments. Furthermore, the presence of polysaccharides in formulations usually increases the viscosity and improves its stability (Campos et al., 2014). Therefore, several common cosmetic ingredients were chosen to be mixed with Cyanoflan, and their effect on the compatibility, stability and viscosity of the mixtures was evaluated at three distinct time points: 0 h, i.e. immediately after preparation (T_0), and after 24 h, with the samples kept at room temperature (RT) (T_{24}), which were then incubated at 40 °C for 6 days (T_{7d}), corresponding to an accelerated stability test.

4.3.1. Evaluation of compatibility and stability

The organoleptic characteristics are of great importance for product acceptance by consumers. Moreover, visual analysis and odor evaluation can provide evidence of physical instability in a formulation. However, such analysis may exhibit variation, since color and general appearance are dependent upon various factors, such as lighting and individual perception, while odor can also vary from person to person and be influenced by odors present in the environment (Patil et al., 2018). Cyanoflan showed to be easily dispersed in water using a homogenizer to decrease the time needed to obtain a homogeneous solution, as commonly performed in the cosmetic industry. Regarding Cyanoflan aqueous solutions, the solution with higher concentration (1% w/v) exhibited a slight yellowish coloration compared to the 0.5% (w/v) one, which displayed near-transparency and minimal chromatic presence (Fig. 4) These characteristics, alongside with the absence of odor and a stable pH (Table 2), remained unchanged for both solutions during the test period of 1 week and with high temperature. Then, distinct formulations using Cyanoflan and common cosmetic ingredients were performed.

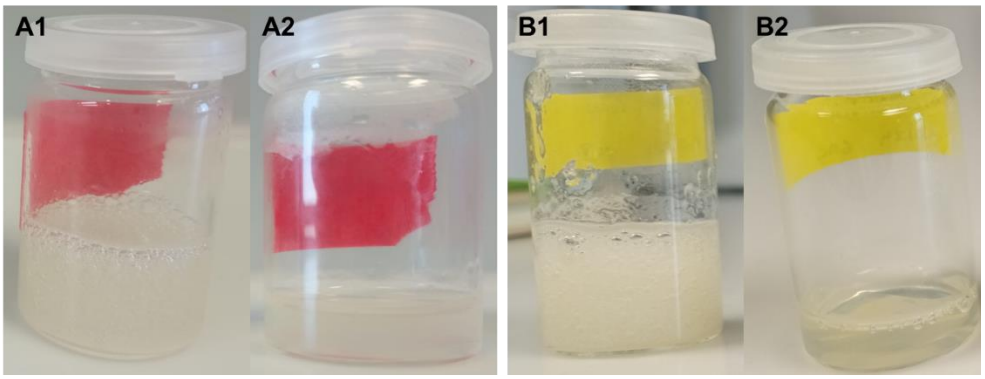

Figure 4. Visual aspect of 0.5% (A) or 1% (B) Cyanoflan (w/v) aqueous solutions at T_0 - immediately after homogenization (A1 and B1, respectively) and T_{7d} - after 7 incubation days (A2 and B2, respectively).

Exfoliators play a crucial role in skin care by removing dead cells from the outermost layer of the epidermis, thereby enhancing the skin's ability to absorb active ingredients present in the cosmetics. Since inorganic salts can be used for that purpose (Bom et al., 2019), magnesium sulphate ($MgSO_4$) was tested in a formulation with Cyanoflan. The biopolymer showed good stability with the salt, given that no significant changes in the formulation regarding pH (Table 2), aspect, color and odor were observed over time.

Table 2. pH values of different formulations after adding distinct cosmetic ingredients to 1% (w/v) Cyanoflan aqueous solution at three different time points: immediately after homogenization (T_0), after 24 h (T_{24}) and 7 days (T_{7d}).

Formulations (w/v)	pH		
	T_0	T_{24}	T_{7d}
0.5% Cyanoflan	10.0	8.0	7.2
1% Cyanoflan	9.1	7.9	8.0
1% Cyanoflan + 2% $MgSO_4$	6.7	5.9	7.3
1% Cyanoflan + 0.1% EDTA	5.6	4.6	4.6
1% Cyanoflan + 5% Glycerin	7.0	7.3	5.8
1% Cyanoflan + 5% Ethanol 96%*	7.0	6.7	5.5
1% Cyanoflan + 10% Lactic Acid*	3.2	3.2	3.2
1% Cyanoflan + 1M NaOH*	9.3	9.2	7.6
1% Cyanoflan + 0.5% SDS	7.6	5.7	7.7
1% Cyanoflan + 0.5% Coco-Glucoside*	6.8	6.3	6.8
1% Cyanoflan + 5% Zinc oxide	8.2	7.4	7.3
1% Cyanoflan + 1% Xanthan gum	6.5	6.6	6.5
0.5% Cyanoflan + 0.5% Carbomer	5.2	5.4	5.4
1% Cyanoflan + 0.5% Carbomer	4.9	4.9	4.9

***** Indicates values in % v/v

The presence of metal residues in cosmetic formulations promotes oxidation reactions that impact the functions of the ingredients; therefore, the addition of chelating agents is crucial for the stability of cosmetic products. Furthermore, chelating agents boost the performance of antioxidants and preservatives in cosmetics (Martins & Marto, 2023). Given this significance, it was important to assess the stability of Cyanoflan with one of the most used chelating agents in the cosmetics industry, EDTA (ethylenediaminetetraacetic acid). On the other hand, solvents play a significant role in several cosmetic products and can be used for a variety of processes, such as extractions, separations, formulations, and synthesis. Organic solvents are predominant, with glycerin and ethanol being among the most frequently utilized (Bom et al., 2019). In the formulations with 1% Cyanoflan (w/v) plus 0.1% EDTA (w/v), 5% Glycerin (w/v) or 5% Ethanol 96% (v/v), no characteristic odor was detected over time and the color remained consistent in all. Furthermore, the pH remained stable throughout the observation period (Table 2).

In cosmetic formulations, pH modifiers are essential to achieve a desired pH of the formulation, which also enhances the product's stability. Cyanoflan was also evaluated in formulations containing pH modifiers, namely, one containing 10% lactic acid (v/v) adjusted to pH 3 and the other containing 1M NaOH (v/v) adjusted to pH 9, both exhibited a persistent yellowish coloration throughout the observation period. No characteristic odor was detected in either formulation. The pH of the lactic acid formulation remained constant over time. On the contrary, a slight alteration in pH was observed in the NaOH formulation at T_{7d} (Table 2). However, this change was deemed insignificant given the stability of the remaining characteristics. The consistent coloration suggests the presence of a stable compound or complex, while the absence of any discernible odor indicates the absence of degradation or contamination. The minor pH variation in the NaOH formulation is unlikely to impact its stability or efficacy, falling within an acceptable range.

Two different surfactants were tested, coco-glucoside, one of the most natural of the chemically produced surfactants available at the moment, produced from fatty alcohol (coconut) and glucose. SDS (sodium dodecyl sulfate) was also tested since is one of the most common synthetic surfactants used in foaming products, to cleanse the skin (Bom et al., 2019). Immediately after the preparation of both formulations (T_0) there was considerable formation of foam in the solutions (Fig. 5 A1 and B1) that was still visible at T_{24}, a phenomenon commonly associated with surfactants due to their ability to reduce surface tension and stabilize interfaces, being commonly used as foaming agents. This surfactant-induced foam formation can enhance the cleansing experience by creating a rich lather, which aids distribute the surfactant evenly over the skin or hair, facilitating the removal of dirt and impurities (Bom et al., 2019). At T_{7d}, there was no longer possible to observe foam in either surfactant solution (Fig. 5 A2 and B2). Both solutions maintained a transparent appearance throughout the observation period, with no apparent changes in odor detected. In both solutions the pH did not suffer any significant alteration. This consistent absence of alterations suggests the stability of Cyanoflan with both the natural and synthetic surfactants.

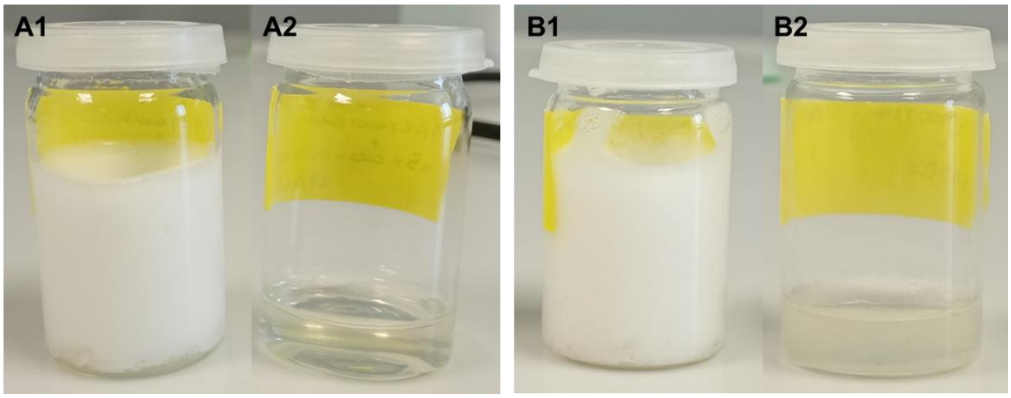

Figure 5. Formulations with surfactants. Visual aspect of (A) 1% Cyanoflan (w/v) + 0.5% coco-glucoside (v/v) at T_0 (A1) and T_{7d} (A2); (B) 1% Cyanoflan (w/v) + 0.5% sodium dodecyl sulphate (w/v) at T_0 (B1) and T_{7d} (B2).

Most cosmetic formulations include colorants in their composition, as they are crucial for enhancing the appearance of cosmetics. Colorants can be either synthetic or natural and are classified as pigments or dyes based on their solubility. Pigments consist of solid, opaque, inorganic particles that are insoluble and dispersed within a vehicle (Guerra et al., 2018). Zinc oxide is commonly used as a pigment, and its mixture with Cyanoflan resulted in a white-colored formulation (Fig. 6), which was maintained until T_{7d}. This observation was in line with the other characteristics analyzed, which also remained unchanged.

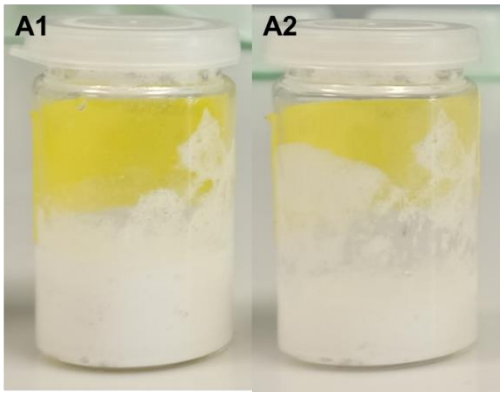

Figure 6. Formulation with pigmentation. Visual aspect of 1% Cyanoflan (w/v) + 5% Zinc oxide (w/v) at T_0 (A1) and T_{7d} (A2).

Thickeners are ingredients that provide consistency to cosmetic products. Most thickeners used in cosmetics are polymers, serving not only as thickeners but also as stabilizers, emulsifiers, and rheology modifiers (Zheng & Loh, 2016). Therefore, it was important to evaluate the stability of Cyanoflan with two distinct types of thickeners, one natural (Xanthan gum) and one synthetic (Carbomer). Similar to the other ingredients, the formulations remained stable in terms of color or odor thoughout the period tested.

In addition, both formulations exhibited a relatively neutral pH. However, it is noteworthy that the aqueous solutions containing Carbomer formed a cloudy acid solution, which was neutralized with a strong base to pH 6, as described by the manufacturer, and formed a mass gel.

4.3.2. Rheological Measurements

The flow curves of apparent viscosity variation (mPa.s), as a function of time (s) and the apparent viscosity variation (mPa.s), as a function of shear rate (s^{-1}) as log-log plot of Cyanoflan with different cosmetic ingredients were compared over three different time points T_0 (Fig. 7), T_{24} (Fig. 8) and T_{7d} (Fig. 9). The apparent viscosity of the 1% Cyanoflan aqueous solution at the lowest shear rate (0.500 s^{-1}) was 5046 mPa.s at T_0. It was also possible to observe a non-Newtonian behavior of Cyanoflan that is verified by the decrease of viscosity as the shear rate increases, named pseudoplastic thinning, as previously observed (Mota et al., 2020). The measurement was conducted again after 24 h at RT (T_{24}), with the apparent viscosity being 4246 mPa.s at the lowest shear rate. All the samples were kept at 40 ºC after the 24 h in order to accelerate the aging process, providing an extrapolation of the long-term behavior of the solutions, i.e. the product shelf life. At T_{7d}, a slight decrease of viscosity was observed for the Cyanoflan solution (1% w/v) reaching 3078 mPa.s. Overall, all the formulations showed the same non-Newtonian behavior with no major alterations over time and the majority showed similar values of apparent viscosity at the lowest shear rate. In addition, the overall constant decrease in viscosity observed at T_{7d} was detected in all the solutions, which suggests some degree of polymer degradation, oxidation, or structural reorganization. In addition, all solutions demonstrated similar viscosity trends, indication of the stability of Cyanoflan when combined with other ingredients. This consistency also implies that Cyanoflan retains its rheological properties under the experimental conditions used. Notably, immediately after homogenization (T_0) and at the lowest shear rate, the formulation containing 1% Cyanoflan (w/v) + 2% $MgSO_4$ (w/v) and the formulation 1% Cyanoflan (w/v) + 0.5% SDS (w/v) exhibited an apparent viscosity of 7108 and 7733 mPa.s, respectively (Fig.7 A1 and B1). Both values higher than the viscosity observed for 1% Cyanoflan (w/v) and all other formulations. However, over time this difference in viscosity gradually decreased, after 24 h (Fig 8 A1 and B1) was less significant, and by T_{7d}, this difference was no longer observable, suggesting a time-dependent equilibration of the system. Remarkably, the solution of 1% Cyanoflan (w/v) plus 10% Lactic Acid (v/v) showed a higher initial viscosity compared to the rest of the solutions in T_{24} (Fig. 8 B1) and T_{7d} (Fig. 9 B1) with values of 9231 mPa.s and 8285 mPa.s, respectively. The increase in viscosity observed in the

presence of lactic acid is probably the result of its interaction with Cyanoflan, potentially leading to the formation of a more robust network. These interactions could involve ionic cross-linking or hydrogen bonding, which are typical in polymeric systems (Lefebvre & Doublier, 2005). Even slight variations in ionic strength or composition can prompt alterations in the physical properties of the formulation. The pH of the solutions, particularly at the higher and lower extremes, also can have an impact since is directly related to the viscosity (Gruber, 1999).

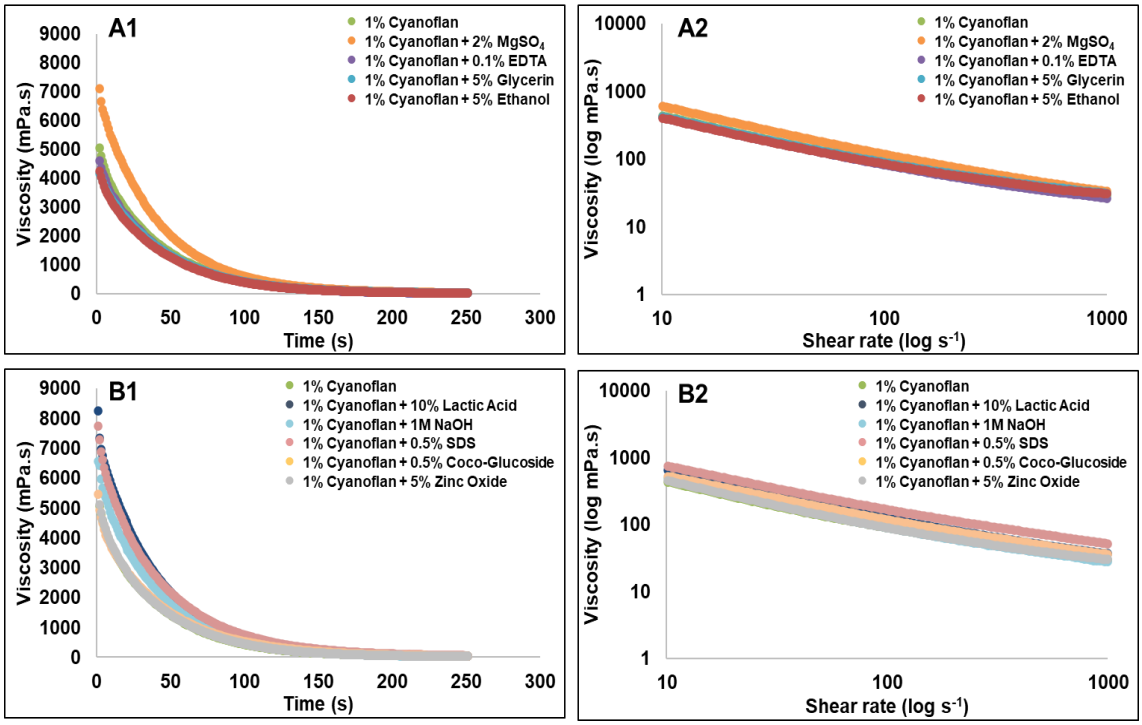

Figure 7. Apparent viscosity in mPa.s as a function of time in seconds (A1 and B1) or as a function of shear rate in log s^{-1} (A2 and B2), of aqueous solution of Cyanoflan with different cosmetic ingredients at T_0 – immediately after homogenization.

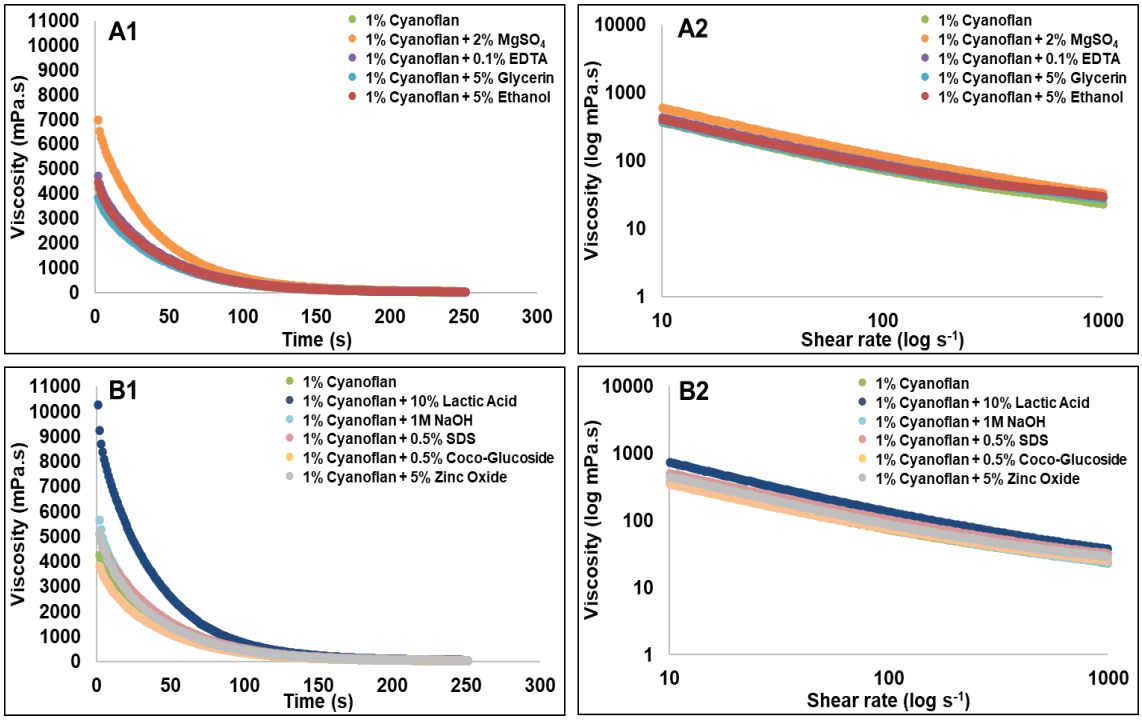

Figure 8. Apparent viscosity in mPa.s as a function of time in seconds (A1 and B1) or as a function of shear rate in log s^{-1} (A2 and B2), of aqueous solution of Cyanoflan with different cosmetic ingredients at T_{24} – 24 h at room temperature after homogenization.

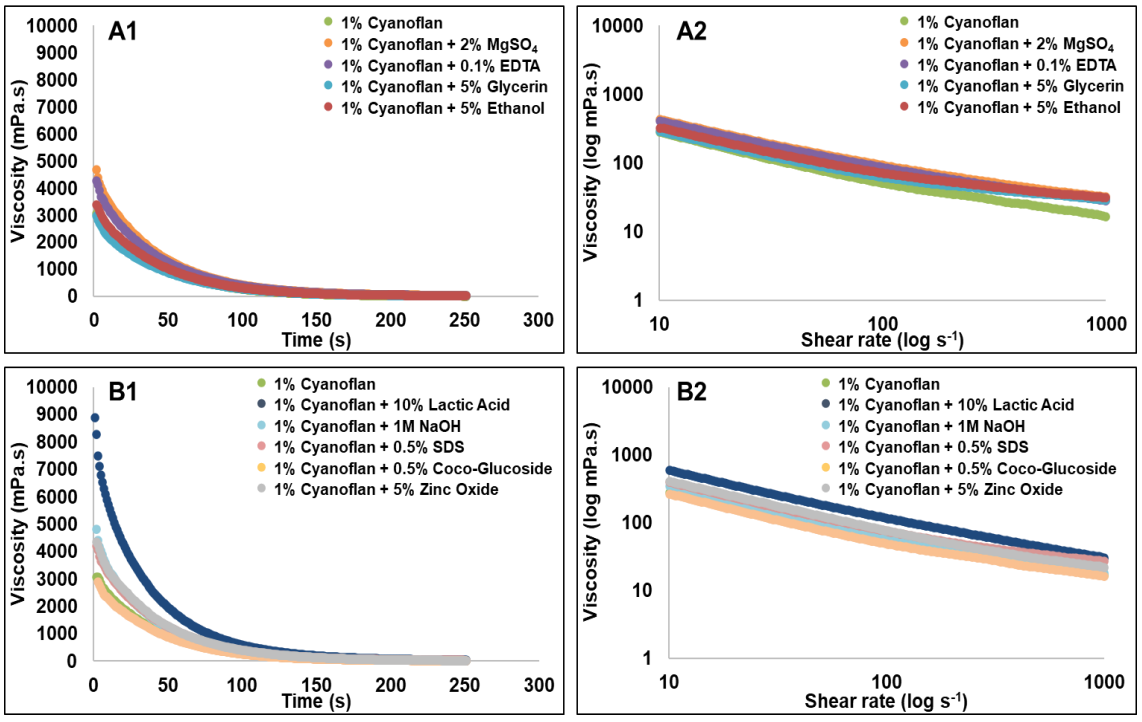

Figure 9. Apparent viscosity in mPa.s as a function of time in seconds (A1 and B1) or as a function of shear rate in log s^{-1} (A2 and B2), of aqueous solution of Cyanoflan with different cosmetic ingredients at T_{7d} – after 7 days incubation.

4.3.2.1 Thickeners

In cosmetic formulations, both natural and synthetic thickeners can be used. Naturally derived thickeners, such as xanthan gum, are polymers that absorb water and swell, increasing the viscosity of formulations. On the other hand, synthetic thickeners, like carbomer, are multifunctional polymers offering benefits like excellent organoleptic properties, low cost, high viscosity at low concentrations, and long-term stability. However, synthetic thickeners can raise sustainability concerns since they are often derived from petrochemicals and may contain impurities (Zheng & Loh, 2016). Therefore, it was important to evaluate the viscosity effects and differences between Cyanoflan and both thickeners.

Xanthan gum is a polysaccharide produced by the bacterium *Xanthomonas* sp. and widely utilized in the cosmetic industry due to its exceptional thickening and stabilizing properties. Even at low concentrations, Xanthan gum can significantly increase the viscosity of liquid formulations, providing desirable rheological properties that improve product application and user experience (Furtado et al., 2022). In Fig. 10 is possible to observe the differences in viscosity at T_0 between the solutions of 1% Cyanoflan (w/v) or Xanthan gum (w/v) alone, and 1% Cyanoflan (w/v) plus 1% Xanthan gum (w/v). Although showing the same non-Newtonian behavior, at a shear rate of 0.500 s^{-1} the 1% aqueous solution of Cyanoflan had an apparent viscosity of 5046 mPa.s, while Xanthan gum demonstrated a higher value of 11497 mPa.s. However, it is important to note the big increase in viscosity of the 1% Cyanoflan (w/v) plus 1% Xanthan gum (w/v) with an apparent viscosity of 24499 mPa.s. This synergistic viscosity has been observed with other polysaccharides like Guar gum (Casas et al., 2000).

The synthetic polymer tested, Carbomer 940, is a fine white powder made from acrylic acid, with a high molecular weight, soluble in both water and alcohol. Its usage typically ranges from 0.1% to 0.5%, depending on the formulation type and the desired final viscosity. It is mildly acidic and, when neutralized to pH 6 forms a loose network (Kolman et al., 2021). The rheological properties of Carbomer, as well as the effects of pH, temperature, and concentration, have been extensively studied, along with its use in cosmetic formulations (Huang et al., 2023; Varges et al., 2019). Regarding our results, immediately after homogenization at T_0, the formulations with Carbomer revealed some differences when comparing the use of synthetic and natural polymers (Fig. 10). Specifically, the apparent viscosity of the formulation incorporating 1% Cyanoflan (w/v) and 0.5% Carbomer (w/v) at a shear rate of 0.500 s^{-1} resulted in a value of 30215 mPa.s,

which is notably higher than the apparent viscosity observed with 1% Cyanoflan (w/v) plus 1% Xanthan gum (w/v). Synthetic polymers, such as Carbomer, often exhibit higher molecular weight and more complex structural configurations than their natural counterparts. These characteristics lead to increased intermolecular interactions and, consequently, a higher resistance to flow under applied shear (Alves et al., 2020). However taking into consideration the environmental and safety advantages of natural polymers, the formulation with Cyanoflan and Xanthan gum still showed a high value of apparent viscosity, which can support their continued research to explore the optimization of these formulations to balance viscosity with other functional properties.

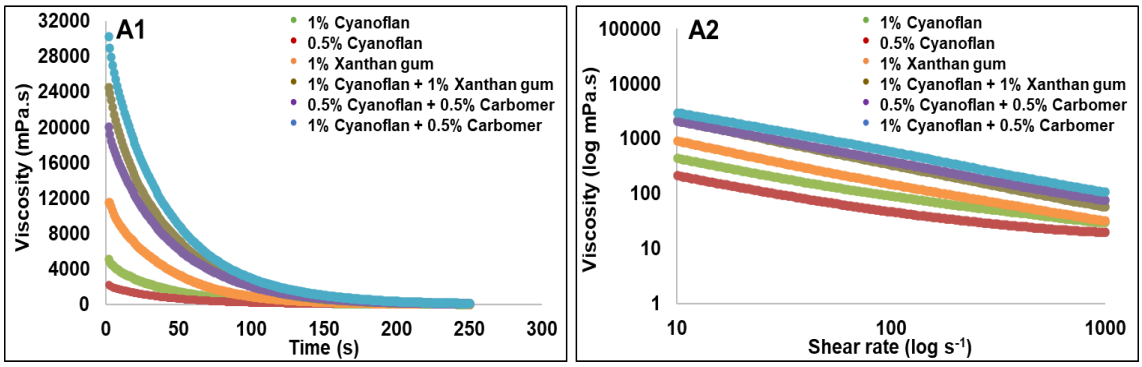

Figure 10. Apparent viscosity in mPa.s as a function of time in seconds (A1) or as a function of shear rate in log s^{-1} (A2), of aqueous solution of Cyanoflan with different thickeners at T_0 - immediately after homogenization.

At T_{24} a considerable decrease in the apparent viscosity was observed in the formulation 1% Cyanoflan (w/v) + 1% Xanthan gum (w/v), which dropped from 24499 to 17647 mPa.s, respectively (Fig. 11). This behaviour is consistent with previously observed results with the other compounds. In contrast, the formulations containing Cyanoflan and Carbomer did not exhibit such a pronounced decrease in viscosity, with the viscosity values of the 1% Cyanoflan (w/v) + 0.5% Carbomer (w/v) formulation, remaining very similar over the 24 h period. However, in the formulation with 0.5% Cyanoflan plus 0.5% Carbomer only a slight decrease in viscosity was observed, from 20013 to 17905 mPa.s. The significant decrease in viscosity may be attributed to natural degradation or reorganization of the polymer network over time. On the other hand, synthetic polymers, in this case Carbomer may have a more robust molecular structure that resists degradation or reorganization under the same conditions.

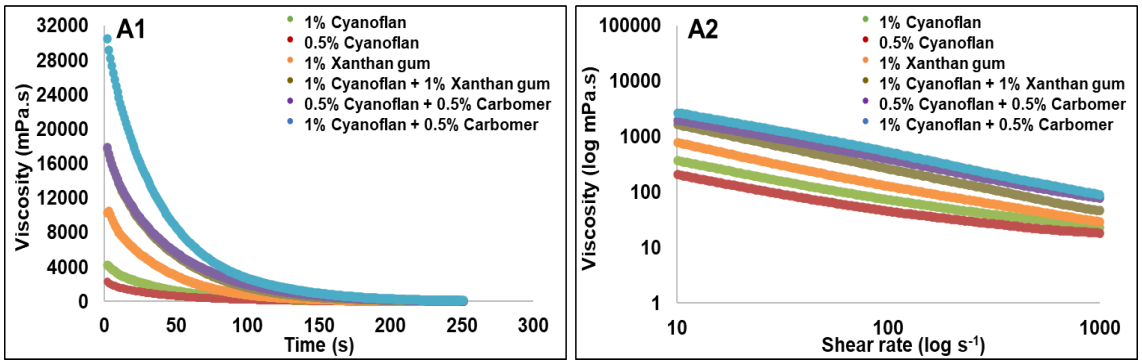

Figure 11. Apparent viscosity in mPa.s as a function of time in seconds (A1) or as a function of shear rate in log s^{-1} (A2), of aqueous solution of Cyanoflan with different thickeners at T_{24} - 24 h at room temperature (RT) after homogenization.

After 7 days of incubation at 40ºC (T_{7d}), even though the apparent viscosity of the formulation of 1% Cyanoflan (w/v) + 0.5% Carbomer (w/v) retained a higher viscosity compared to the other formulations, it was possible to visualize a higher decreased over time, similar to the other formulations. Additionally, the formulation with 0.5% Cyanoflan (w/v) plus 0.5% Carbomer exhibited nearly the same viscosity value as the 1% Cyanoflan + 1% Xanthan gum (w/v), 11343 mPa.s and 12406 mPa.s respectively (Fig. 12). Based on these results, the mixture of the two natural polysaccharides provides a promising way to achieve high viscosities while potentially reducing the overall usage of synthetic ones. It is not only enabling more sustainable and cost-effective industrial processes, but also encourages further research into the unique properties and applications of cyanobacterial EPS.

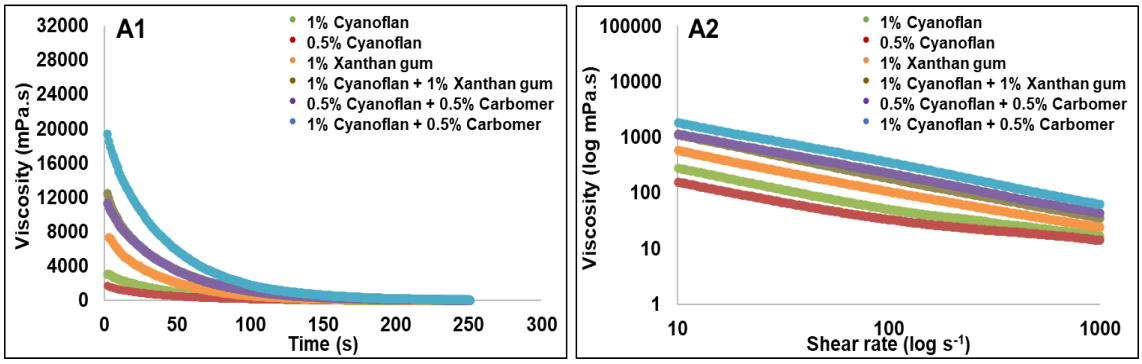

Figure 12. Apparent viscosity in mPa.s as a function of time in seconds (A1) or as a function of shear rate in log s^{-1} (A2), of aqueous solution of Cyanoflan with different thickeners at T_{7d} - after 7 days incubation.

5. Conclusion

In the present work, it was possible to gain new insights on the natural extracellular cyanobacterial polymer, Cyanoflan, regarding its antioxidant activity as well as its compatibility with other ingredients commonly used in cosmetic formulations. The total antioxidant capacity values obtained were comparable to other natural polysaccharides, setting a precedent for future evaluation of other antioxidant properties, thereby conferring additional features to Cyanoflan. Stability studies demonstrated that Cyanoflan maintained excellent stability in terms of pH, color, and odor when combined with various common formulation ingredients across all time points tested. This result confirms Cyanoflan's compatibility in diverse formulations, even at high temperature. Rheological assessments further supported Cyanoflan’s potential as a cosmetic ingredient, as all formulations showed non-Newtonian behavior and maintained approximate values of apparent viscosity among the different formulations. Notably, mixtures with other thickeners, including the one of natural origin (Xanthan gum) exhibited high viscosity, underscoring promising synergistic effects. These results are a significant step toward establishing Cyanoflan as an effective rheology modifier and thickener with antioxidant activity, indicating its potential utility as a multifunctional natural ingredient in cosmetic formulations.

6. Future Perspectives

Future research should focus on expanding the understanding and application of the cyanobacterial polymer Cyanoflan as a natural bioactive rheological modifier in cosmetic formulations. For this purpose, it would be necessary to explore the sustainable and scalable production of Cyanoflan, which can be fundamental for its commercial viability, including the optimization of cultivation conditions, extraction process and purification techniques, to ensure a consistent and cost-effective product. Additionally, cyanobacterial biomass could be used to extract bioactive compounds or valuable by-products (e.g. phycocyanin).

Another step could be to evaluate the antioxidant potential by other chemical methods, such as Ferric Reducing Antioxidant Power (FRAP) and Oxygen Radical Absorbance Capacity (ORAC), which could help validating Cyanoflan not only as a rheological modifier, but also as an active ingredient contributing to the overall skin health benefits in cosmetic or cosmeceutical products. Furthermore, investigating Cyanoflan's ability to inhibit enzymes relevant to skin aging (e.g. collagenase and elastase) could help establishing Cyanoflan as a potential ingredient in anti-aging formulations. Additionally, the rheological studies on more complex and varied cosmetic formulations could be further pursued, possibly testing formulations with more than one ingredient, at different concentrations, and/or under different conditions (e.g. pH and temperature) to ensure stability and performance consistency. In a final phase, it could be important to conduct market research studies to understand consumer preferences and general acceptance of natural polymers in cosmetics.

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