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Investigation of natural-derived materials as substitutes for synthetic ingredients in cosmetic formulations

Surname: **Spena**

Name: **Francesca**

Registration number: **876028**

Tutor: Prof. Miriam Colombo

Co-tutor: Dr. Marco Davide Giustra

Supervisor: Prof. Paolo Galli

Coordinator: Prof. Paolo Galli

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List of Abbreviations

Abbreviation	**Meaning**
ATR	Attenuated Total Reflectance
CMC	Carboxymethyl Cellulose
CO_2	Carbon Dioxide
DMEM	Dulbecco’s Modified Eagle Medium
DMF	Dimethylformamide
DoE	Design of Experiments
EMEM	Eagle’s Minimum Essential Medium
EtOH	Ethanol
EU	European Union
FBS	Fetal Bovine Serum
FT-IR	Fourier Transform Infrared Spectroscopy
HCl	Hydrochloric Acid
HDPE	High density Polyethylene
IC_{50}	Half Maximal Inhibitory Concentration
LDPE	Low -density Polyethylene
LLDPE	Linear Low -density Polyethylene
MEM	Minimum Essential Medium
MP	Microplastic
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
PE	Polyethylene
PMMA	Poly (methyl methacrylate)
PET	Polyethylene terephthalate
PMW	Poly (isobutylene-alt-maleic anhydride)-tryptophan derivative
PP	Polypropylene
PU	Polyurethane
RPMI	Roswell Park Memorial Institute medium
SEM	Scanning Electron Microscopy
SPF	Sun Protection Factor
UV	Ultraviolet
UVA	Ultraviolet A
UVB	Ultraviolet B
UVC	Ultraviolet C

Abstract

With the increasing demand for environmentally safe cosmetic products driven by regulatory restrictions and growing public awareness of the environmental impact of microplastics, the development of biodegradable alternatives from renewable resources has become a major research focus. Carbohydrate-based polymers represent particularly attractive candidates due to their biodegradability, abundance, renewability, and structural versatility. In this work, microparticles based on starch, chitosan, and carboxymethylcellulose were produced using different processing and chemical modification strategies to obtain micrometric particles with tailored structural and rheological properties.

In parallel, a formulation strategy was developed to establish a sustainable Pickering emulsion platform capable of incorporating and exploiting the functional properties of the synthesized biodegradable microparticles. Microparticles derived from native and chemically modified tapioca starch exhibited good compatibility with the optimized emulsion system, while preserving pseudoplastic flow behavior and inducing controlled modifications of the droplet size distribution. These results demonstrate that starch-based microparticles can effectively act as co-stabilizers and structure-modifying agents in sustainable cosmetic emulsions.

In contrast, chitosan- and carboxymethylcellulose-based microparticles did not provide satisfactory texture or stabilization under the investigated conditions, indicating the need for further optimization and deeper investigation of their interfacial behavior and formulation compatibility. Overall, the results highlight the potential of modified polysaccharide microparticles, especially those based on starch, as effective biodegradable substitutes for traditional microplastics. This allows for high-performance cosmetic products that meet environmental sustainability standards and regulatory demands.

Introduction

1. Microplastics in Cosmetics: sources, functional applications, and major polymer classes

In recent years, the cosmetics market has gained prominence, reflecting not only market growth but also an ecological transition, with economic policies encouraging the adoption of sustainable and biodegradable materials [1]. The eco-friendly cosmetics market is expected to reach $5.25 billion by 2029, with the global cosmetics market ranking roughly third in terms of fastest growth [2]. Plastics and microplastics production is currently causing one of the most serious environmental problems, especially those related to the cosmetics industry. Waste plastics, the textile industry, and cosmetics are among the many sources of microplastics [1]. Microplastics were first identified in the environment in the early 1970s, when small plastic fragments were detected in the Sargasso Sea [3]. The term microplastic was later introduced in the early 2000s to describe plastic particles observed in marine sediments, marking the beginning of systematic research on this class of contaminants [4]. In 2008, an expert workshop convened by the National Oceanic and Atmospheric Administration (NOAA) established a widely adopted definition of microplastics as plastic particles smaller than 5 mm[5].

Recent classifications have suggested smaller size ranges, usually between 1 μm and 1 mm. Microplastics are generally characterized as solid, synthetic polymeric particles that are insoluble in water at ambient temperature and may exhibit a variety of shapes, including fibers, fragments, films, and microbeads [6]. Microplastics are composed primarily of carbon and hydrogen atoms linked in polymer chains [7].

It's crucial to distinguish microplastics from smaller plastic particles, such as submicron plastics and nanoplastics, which have distinct physicochemical properties but are expected to share similar environmental pathways and fate [8].

Microplastics can be found in a variety of sources, including industrial applications and everyday consumer products. Some of the most common sources of microplastics include: [9]

- **Textiles**: Synthetic fabrics such as nylon, polyester, and acrylic release microfibers during washing.
- **Synthetic paints and tire wear**: The abrasion of road markings, tires, fishing equipment, aquaculture gear, and painted surfaces generates significant quantities of microplastics.
- **Macroplastic debris in the environment**: Larger plastic items, including bottles, bags, and packaging materials, degrade over time through photodegradation and environmental weathering.

- **Personal care products**: Microbeads used in exfoliating scrubs, toothpaste, and other cosmetic or hygiene products.
- **Industrial raw materials**: Plastic pellets and other polymer intermediates employed in manufacturing processes can be released into the environment, representing an additional source of microplastic pollution.

Based on their origin, microplastics are commonly classified into two main categories: primary and secondary microplastics (**Figure 1.1**) [10]. Primary microplastics are intentionally manufactured at the microscopic scale for specific uses, such as formulation components, exfoliating agents, whereas secondary microplastics result from the fragmentation and degradation of larger plastic items through physical, chemical, and biological processes [1].

Figure 1.1 Microplastics originating from personal care and cosmetic products can disseminate throughout environmental compartments, including terrestrial and marine ecosystems, ultimately leading to human exposure. These particles have been detected in multiple human tissues and organs [10].

Cosmetic formulations are designed for application to the external surfaces of the human body with the primary objectives of maintaining physiological condition, altering or enhancing appearance, providing protection against environmental stressors, and controlling body odor. A more detailed functional classification categorizes cosmetic products into several groups, including: (1) personal hygiene and cleansing products, such as shampoos, soaps, and deodorants; (2) skin and hair care products intended for topical application; (3) decorative or beautifying products, including lip products and fragrances; (4) protective formulations, such as sunscreens and anti-aging treatments; (5) corrective products, including hair colorants and facial masks; (6) maintenance products, such as moisturizers and shaving preparations; and (7) products containing active agents, for example antiseptic formulations and fluoride-containing toothpastes [11].

Cosmetic products are divided into two types depending on their duration of use on the human body: rinse-off and leave-on products. Rinse-off products include those intended to remain in contact a short period with the skin, hair or mucous membranes to achieve their purpose. Typical examples include shampoos, toothpaste, liquid soap, shaving foam and bath/shower additives. Leave-on products, such as skin care formulations, make-up, and nail varnish are designed to maintain prolonged contact with the body [12]. Personal care products, including cleansing products, makeup cosmetics, shower gel, facial cleanser, handsanitizer, soap, toothpaste, sunscreen and shampoo play a significant role in the environment as sources of microplastics [13]. Prior to the advent of microplastics, cosmetic production has been using natural compounds since ancient times. Sugar, cellulose, starch, proteins, fats, and natural oils were processed by the chemical industry in different fields. In the 1980s, microplastics replaced these natural ingredients in many products, both in formulations and packaging of cosmetic products due to their versatility [14].

Initially, plastic microspheres were incorporated into cleansing and exfoliating products due to their gentle abrasive properties, resulting in effective but non-aggressive exfoliation, associated with very high skin compatibility. Over time, their application in cosmetics has progressively expanded into a wide range of functions, including film-forming agents, thickeners, viscosity regulators, or emulsion stabilizers, enhancing formula stability and prolonging shelf life [15]. Due to their highly tunable particle size, shape, density, and chemical composition, synthetic polymeric powders have enabled significant improvements in spreadability, softness, optical blurring effects, and formulation stability. These microplastics are specially designed and optimized in a spherical form to minimize scratches on the skin and prevent the natural skin growth process. Microplastics enhance texture and spreadability in creams, lotions, or makeup formulations, and achieve smooth and uniform sensory performance while being applied. They possess beneficial physicochemical properties, including being chemically inert, odorless, and not sensitizing or irritating skin [14]. Cosmetic powders are a crucial functional ingredient in cosmetic formulations due to their broad applicability and ability to modulate both sensorial performance and skin-related effects. Their physicochemical properties, including particle size, morphology, surface chemistry, and porosity, enable a wide range of functionalities, such as mechanical exfoliation through the removal of superficial corneocytes, fixation and stabilization of semi-solid formulations, control of light reflection through mattifying or brightening effects, and sebum absorption [14] for oil regulation, particularly in areas prone to excess sebaceous activity (**Figure 1.2**).

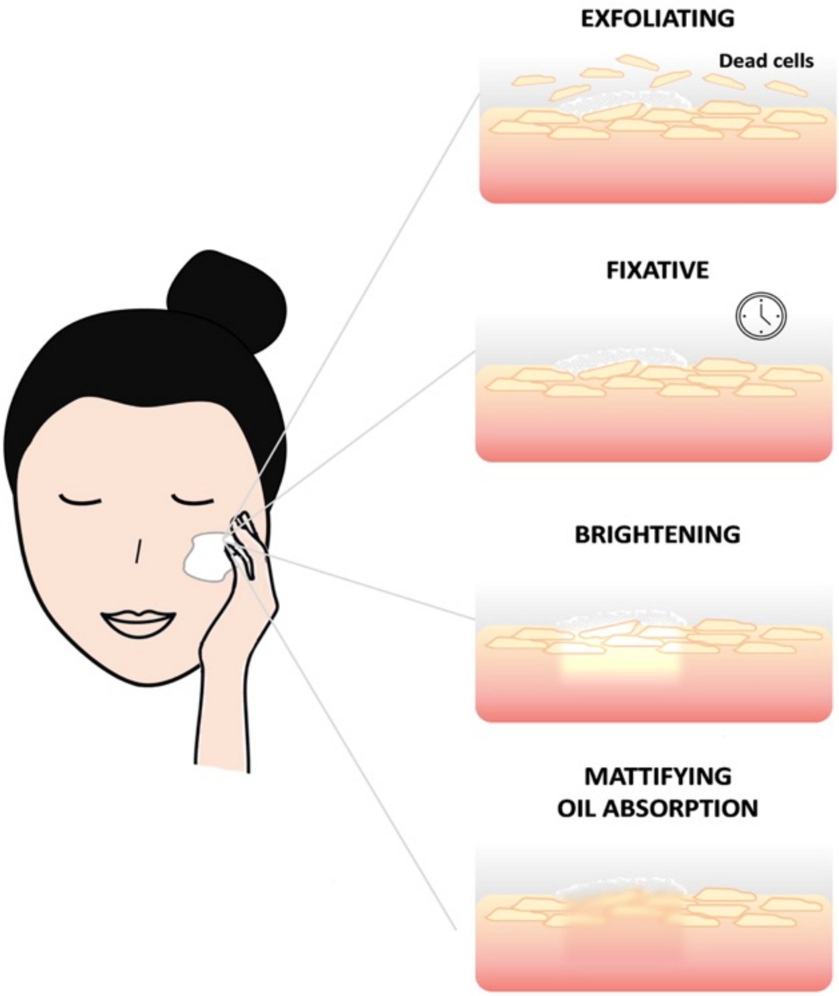

Figure 1.2 Main functional properties of cosmetic powders in formulations and their corresponding effects on the skin: exfoliating, fixative, brightening, mattifying and oil absorption [14].

These attributes allow cosmetic powders to be combined into multifunctional formulations designed to set makeup, improve skin texture, enhance facial definition, and prolong product wear.

Polyethylene microspheres are the most prevalent use, but there is a variety of other applications that include polyurethane, polypropylene, polyethylene terephthalate, polymethyl methacrylate, and nylon [16] (**Table 1**).

- Polyethylene (PE) is among the most extensively used synthetic polymers and is easy to produce. PE is composed of repeating ethylene units that confer flexibility and mechanical resistance. Indeed, the relative polymerization can yield branched or linear structures, leading to distinct molecular architectures, resulting in high-density polyethylene (HDPE), low-density polyethylene (LDPE), and linear low-density polyethylene (LLDPE), each characterized by specific physical and functional properties. In cosmetics, PE is primarily employed as a film-forming and viscosity-modifying agent. PE microparticles were used in formulations for facial and body scrubs as abrasive exfoliants; however, since 2018, their use in cosmetic products has been progressively restricted due to their impact on marine ecosystems.

- Polyurethane (PU) is a synthetic polymer obtained from the reaction between polyols and diisocyanates. In cosmetics, PU microbeads have been utilized as exfoliating, texture enhancement, and/or binding agents. Their capacity to absorb excess sebum makes them particularly suitable for oil-control products.
- Polypropylene (PP) is a thermoplastic homopolymer synthesized from 1-propene, a gas derived from petroleum and natural gas. Due to its low density and high resistance to chemical, mechanical, and moisture-induced degradation, PP has found application in cosmetics as a rheology-modifying agent in products such as foundations and nail lacquers, as well as an exfoliating component in selected formulations.
- Polyethylene Terephthalate (PET) is derived from terephthalic acid and ethylene glycol. PET microparticles can be utilized in various formulations to enhance the texture of semi-solid formulations and serve as exfoliating agents in skincare products like face and body scrubs. Furthemore, they can function as mattifying agents in creams, lotions, and foundations.
- Poly (methyl methacrylate) or PMMA is a transparent thermo-plastic polymer derived from methyl methacrylate. In cosmetics, this polymer is used in in decorative products such as nail polishes, lip glosses, and mascaras due to its excellent film-forming properties. PMMA micro-particles are also used as absorbent agents for transporting active ingredients and as brightness/mattifying regulators, that help minimize the appearance of age-related skin imperfections. Their fine particle size further enables modulation of formulation texture and sensorial performance.
- Polyamides, commonly referred to as nylons, are synthetic polymers containing amide bonds as repeating units, and various types can be obtained depending on the precursor monomers; eminent examples are Nylon-6 and Nylon-12. Nylon-6 is employed as a viscosity regulator, bulking agent, and moisturizing component. Nylon-12 exhibits pronounced bulking and opacifying properties and is frequently incorporated into skin creams and face powders [14].

Table 1. Predominant microplastic polymers

Plastic Type	Size Range	Enviromental Impact	Health impact	References
Polyethylene	0.06 to 11.06 µm	Contribute to marine pollution, harms marine life	Potential ingestion through contaminated sea food, respiratory issues	[17], [18]
Polyurethane	Less than 5 mm	Pollutes land and water, poses risks to terrestrial ecosystems	Respiratory problems, potential skin irritation, potential effects on immune and nervous systems	[19], [20]
Polypropylene	120 to 220 µm	Poses risks to wildlife	Gastrointestinal problems, respiratory issues	[21], [22]
Polyethylene Terephtalate (PET)	Microfiber dimensions: 12 to 18 µm in thickness Lenght: shorter than 1 mm	Contaminates water bodies, harms marine animals	Ingestion through contaminated sea food, potential respiratory issues	[23], [24]
Poly (methyl methacrylate) or PMMA	10 to 50 µm	Poses risks to marine ecosystems	Ingestion may induce oxidative stress and inflammation,inhalation of smaller particles may cause pulmonary inflammation and cellular stress	[25]
Nylon	Fibers: 5 µm – 5 mm Fragments in the 1–100 µm range	Major contributor to microfiber pollution, widely detected in marine and freshwater environments	Ingestion through contaminated food and water,inhalation of airborne fibers may cause respiratory irritation	[26]

These microbeads can make up to 10% of the product's weight and can reach several thousand particles per gram of the product [27]. From an industrial point of view, the relatively low cost of microplastic raw materials represents an additional advantage, as it contributes to cost-effective formulation strategies and improved economic profitability for cosmetic manufacturers.

Determining the amount of MPs released by various sources and nations is a challenge, and current estimates are not always accurate. In 2012, the European Union (EU) generated 4360 tons of primary MPs for use in cosmetics [16].

1.1. Environmental and human health impacts of microplastics

Although microplastics improve several product features, such as durability and sensorial properties, they are not environmentally friendly. Their high environmental persistence usually results in accumulation within aquatic ecosystems, which can threaten marine life and, indirectly, human health [28]. The production of microplastics has significantly risen and the concentrations detected on the coasts of certain marine areas can reach thousands of particles per cubic meter. These numbers are predicted to double in the next few years without proper measures [29]. Microplastics are characterized by high chemical stability and inability to degrade, allowing them to persist in the environment, meaning they can persist in the environment for decades [30]. Their environmental life cycle, which involves processes of transport and bioaccumulation, originates from the release of primary or secondary microplastics into terrestrial and aquatic environments, followed by their mobilization and transfer into freshwater and marine systems. Indeed, MPs from cosmetic and personal care products are able to enter wastewater streams and subsequently pass through sewage treatment plants into aquatic and marine environments [31]. Conventional wastewater treatment processes have been shown to have limited efficiency in the complete removal of MPs, allowing a portion of these particles to pass through the filtration stages and be released into natural ecosystems [32]. They can be found in the ocean, soil, and even the air we breathe. Given the absence of effective strategies for removing microplastic contaminants from marine environments and their high resistance to degradation processes, microplastic particles can be readily adsorbed or ingested by a wide range of marine organisms. The uptake of these particles by marine fauna, plankton, and other biota leads to adverse effects that propagate throughout the entire marine food chain. The presence of microplastics has already been reported in several marine organisms, including copepods [33], bivalves [34], fish [25], and seabirds [36]. The consumption of these pollutants has been proven to have toxic effects on aquatic life, including fish, oysters, mussels, and sea turtles, which can compromise their immune and digestive systems and potentially lead to their demise [37]. Microplastics have the potential to be endocrine disruptors, affecting the hormonal signaling pathways that are crucial for the proper functioning of reproductive systems [38]. To maintain the biodiversity and balance of marine ecosystems, it is crucial to preserve the reproductive health of marine animals. The impact of microplastics on this aspect of animal physiology represents a significant hazard to the well-being of these systems.

Microplastics can undergo aging processes in soil environments through interactions with soil microorganisms and their metabolic activity [39]. Microbial communities may persist on the surface of microplastics for extended periods, leading to the formation of biofilms that can alter the physicochemical characteristics of the particles. These interactions can induce significant modifications in the local microbial community and soil properties [40]. Given the extensive distribution of microorganisms and the propensity of microplastics for fragmentation and migration, close and widespread interactions may occur within shared microenvironments. Nevertheless, the underlying mechanisms and the resulting ecological consequences of these processes remain poorly understood.

Another critical issue is related to the large specific surface area of microplastics, which improves their capacity to adsorb hydrophobic chemicals (e.g., polychlorinated biphenyls) from aquatic environments. Consequently, microplastics may act as vectors for other hazardous contaminants, including heavy metals (Al, Cd, Co, Cr, Cu, Hg, Mn, and Pb), hydrophobic organic compounds, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pesticides, and persistent organic pollutants [41], [42]. Moreover, microplastic particles smaller than 130 µm can enter the human body through ingestion, inhalation, or dermal absorption and subsequently translocate into the bloodstream, lymphatic system, and internal organs, where they may accumulate in various biological matrices, including blood, breast milk, and placental tissue [43]. Once microplastics enter our bodies, they trigger inflammation, contributing to a wide range of adverse health issues [34].

Microplastics may act as vectors for the release and transport of toxic chemical compounds, leading to a range of health problems, including cancer [45], reproductive problems, and developmental issues [46]. Emerging evidence further indicates that microplastics may contribute to the growth of antibiotic resistance, because their surfaces can serve as a proliferation field for bacteria, facilitate bacterial persistence and the acquisition of antibiotic-resistant traits over time.

Recent studies have increasingly demonstrated the presence of MP in multiple human organs and biological matrices, raising significant concerns regarding their potential impacts on human health. (**Figure 1.1.1**) [14].

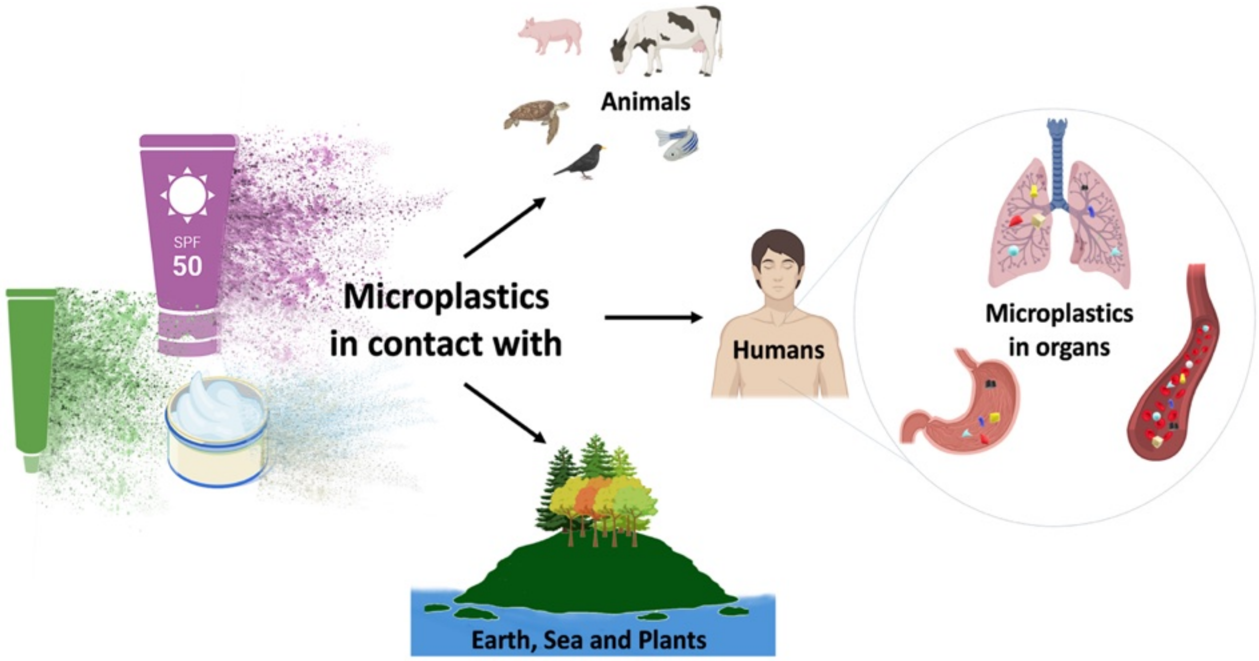

Figure 1.1.1 Microplastics originating from personal care and cosmetic products can disseminate throughout environmental compartments, including terrestrial and marine ecosystems, ultimately leading to human exposure. These particles have been detected in multiple human tissues and organs [14].

Evidence of MPs in human blood has confirmed their ability to cross biological barriers and circulate systemically, suggesting that absorption may occur at a faster rate than elimination through physiological pathways such as renal or biliary excretion. Although the long-term effects of MPs in the bloodstream remain still unknown, their systemic distribution emphasizes the need to assess potential effects on cardiovascular and physiological functions. Beyond circulation, MPs have been detected in cardiovascular tissues, including the human heart and venous tissue, indicating their capacity to accumulate in critical organs [47]. The presence of MPs in cardiac tissues suggests possible interactions between medical interventions and MP exposure, which may have implications for post-operative recovery and cardiovascular health. Similarly, the detection of MPs in vein tissues further supports their persistence within the vascular system [48].

Emerging evidence has also identified MP contamination in the human male reproductive system. The presence of MPs in sperm and testicular tissues demonstrates their ability to reach and accumulate in reproductive organs [49].

These findings raise concerns about potential risks to male reproductive health and fertility, despite the need for further investigation into the underlying mechanisms and long-term effects. A major issue is the presence of MPs in the human placenta, an organ essential for regulating maternal–fetal exchange. Multiple studies have confirmed that MPs of varying sizes and polymer compositions can localize in different placental compartments, including both intracellular and extracellular regions. This accumulation suggests that MPs may interfere with placental function and pose potential risks to fetal development, emphasizing the importance of understanding maternal exposure pathways and transplacental transport mechanisms [50], [51].

In addition, the respiratory and gastrointestinal systems, recognized as primary entry routes for MPs, have been proven to hold microplastic particles. MPs detected in lung tissues and sputum samples provide strong evidence that inhalation represents a significant exposure pathway, particularly in association with environmental factors, smoking habits, and medical procedures [52]. Recent evidence suggests that microplastics can localize within the human renal system. MPs have been identified in urine samples from individuals living in different urban environments, demonstrating that small-sized, pigmented microplastic fragments can be excreted through the urinary pathway. Furthermore, the detection of MPs in healthy human kidney tissues is clear evidence of microplastic deposition within renal structures. These findings suggest that, while renal excretion may represent one elimination route, the kidneys themselves may serve as sites of MP accumulation, highlighting the possibility of potential nephrotoxic effects [53], [54]. These findings indicate that MPs can enter the human body through multiple routes, distribute across various organs, and persist in sensitive tissues, underscoring the urgent need for coordinated actions, as well as changes and optimizations of production processes and products in the cosmetics industry.

1.2. Regulatory landscape and current bans on microplastics in cosmetics

As a response to the growing amount of evidence that highlights the environmental and human health risks associated with plastic and microplastic pollution, regulatory actions and policy initiatives targeting marine litter and microplastics have progressively emerged at both national and international levels. The early awareness of plastic pollution led to the introduction of the first legislative measures to reduce marine litter, culminating in the adoption of the Microbead-Free Waters Act in 2015, which represented the first explicit ban on microbeads. This achievement set the stage for future restrictions on the intentional use of microplastics in cosmetic and personal care products in multiple jurisdictions.

Parallel to regulatory developments, non-governmental organizations and advocacy campaigns have played a crucial role in raising public awareness of microplastics in cosmetics. Initiatives led by organizations such as Greenpeace, Friends of the Earth, and consumer-oriented campaigns have not only highlighted the environmental consequences of microplastic use but have also drawn attention to misleading sustainability claims, commonly referred to as “greenwashing” [55]. This term describes practices in which environmental responsibility is promoted primarily for marketing purposes rather than reflecting genuine sustainability commitments. The European Union's regulatory bodies have begun to address greenwashing through consumer protection frameworks as a response to this. Although there have been recent directives aimed at improving transparency and preventing unfair commercial practices, regulations regarding cosmetics are still limited.

At the same time, increased consumer awareness has driven demand for cosmetic products marketed as environmentally responsible, cruelty-free, and free from specific synthetic ingredients, alongside a growing preference for sustainable or recycled packaging [56]. While this shift has resulted in industry adaptation, it has also highlighted the need for clarity and harmonized regulatory standards to distinguish substantiated environmental claims from misleading ones.

Globally, efforts to regulate microplastics have intensified over the past two decades. International conventions and United Nations initiatives have progressively recognized plastics as a major contributor to marine pollution, emphasizing that the majority of plastic waste originates from land-based sources and that wastewater treatment systems are insufficient to effectively remove microplastics. These concerns have informed policy frameworks such as the European Green Deal and United Nations action plans, which promote reduction strategies for microplastic emissions [14]. In this context, regulatory agencies have proposed standardized definitions of microplastics and restrictions on their intentional use, particularly in consumer products.

Europe has emerged as a leading region in the implementation of microplastic regulations, with several countries introducing early bans on microbeads and The European Union is moving in the direction of comprehensive bans on intentionally added microplastics in cosmetics, personal care products, and certain single-use applications. The Circular Economy Action Plan incorporates these measures, which aim to significantly reduce microplastic releases into the environment by 2030 [57].

In January 2019, the European Chemicals Agency (ECHA) put forward a proposal to restrict the intentional use of microplastic particles in consumer and professional mixtures throughout the European Union, with the projected goal of reducing environmental microplastic emissions by approximately 400,000 tonnes over a 20-year period [58]. Since January 1, 2020, Italy has prohibited the advertising of cosmetic products with MP that can be rinsed off and have an exfoliating or cleansing function [59]. Similar regulatory trajectories have been observed in North and South America, [60] as well as in parts of Asia and Oceania [61], [62] where bans on microbeads in rinse-off cosmetics and personal care products have been implemented at national or regional levels. In addition to governmental regulations, several private sector initiatives have emerged to mitigate microplastic pollution. Many companies have committed to reducing single-use plastics and implementing recycling programs to limit plastic waste. While these efforts represent a step in the right direction, substantial work remains to reduce environmental microplastic contamination. Coordinated action by governments, industry, and individuals, including minimizing plastic use and ensuring proper waste management, is essential to safeguard ecosystems and human health [63]. Effectively addressing microplastic pollution, however, presents multiple challenges. Public awareness remains limited, with many individuals unaware of the extent of microplastic contamination and its potential impacts on environmental and human health. Raising knowledge, particularly among students and future stakeholders globally, is critical to foster societal engagement and political will. Furthermore, the issue is inherently complex, requiring multifaceted solutions that involve coordinated efforts across governments, industries, and communities, as well as systemic changes in production, consumption, and behavioral patterns.

However, despite increasing global convergence on the definition and size range of microbeads, regulatory approaches remain heterogeneous and a universally standardized framework governing the restriction of microplastics in cosmetic formulations is still lacking. Overall, the progressive introduction of bans and regulatory measures reflects growing recognition of microplastics as an emerging environmental and public health concern. Nevertheless, the absence of globally standardized regulations underscores the need for continued international cooperation, robust scientific evidence, and transparent regulatory frameworks to effectively address microplastic pollution and its implications for both ecosystem and human health.

1.3. Other synthetic ingredients of concern in cosmetic formulations

In addition to microplastics, several other classes of synthetic ingredients commonly used in cosmetic formulations are currently facing increasing concern due to their environmental persistence, potential toxicity, and regulatory restrictions. Several studies have documented the presence of preservatives such as parabens, plasticizers including phthalates, formaldehyde-releasing agents, ultraviolet (UV) filters, synthetic fragrances, and heavy metals in commercially available cosmetic products [64].

Among these, preservatives such as parabens and formaldehyde-releasing agents have raised concerns due to their potential endocrine-disrupting effects and skin sensitization. Parabens, in particular, are among the most extensively studied because of their widespread use, with reported concentrations reaching up to 7860 mg/kg in commonly used skincare products, indicating significant exposure through routine application. Biomonitoring and toxicological studies have further linked paraben exposure to hormonal imbalances, reproductive toxicity, and metabolic disorders.

Phthalates and synthetic fragrance compounds constitute another relevant category of concern. Commonly used to enhance fragrance persistence and improve product flexibility, these substances are often present as undeclared ingredients, reflecting discrepancies in labeling practices. Their chronic exposure has been associated with endocrine disruption, allergic responses, and developmental toxicity, particularly in the context of cumulative, multi-product use [65], [66]. Heavy metals also represent a critical toxicological issue in cosmetic formulations. Elements such as lead, cadmium, mercury, nickel, and arsenic have been detected in a wide range of products, including lipsticks, foundations, and eye cosmetics, with higher concentrations often reported in poorly regulated markets. Their presence raises concerns due to well-established neurotoxic, carcinogenic, and systemic health effects [64].

In addition to these contaminants, several classes of widely used synthetic ingredients contribute to environmental persistence and potential health risks. Silicone-based polymers, valued for their film-forming and conditioning properties, exhibit low biodegradability and a tendency to accumulate in aquatic environments. Similarly, petroleum-derived ingredients such as mineral oils and petrolatum remain extensively employed as emollients, despite their non-renewable origin and limited alignment with sustainability goals. Synthetic surfactants, including sodium lauryl sulfate and sodium laureth sulfate, have been associated with skin irritation and ecotoxicological effects, while antimicrobial agents such as triclosan raise further concerns regarding human health and environmental impact.

Furthermore, non-biodegradable synthetic polymers, such as carbomers and acrylate-based copolymers, are widely used as rheology modifiers and film-forming agents, contributing to environmental persistence in a manner comparable to microplastics. Alongside emerging contaminants such as per- and polyfluoroalkyl substances (PFAS) and certain ultraviolet (UV) filters, these compounds are characterized by resistance to degradation, bioaccumulation potential, and possible endocrine-disrupting effects [67].

Overall, these considerations highlight that the replacement of microplastics represents only one aspect of a broader transition toward safer and more sustainable cosmetic formulations based on biodegradable and bio-based materials.

1.4. Eco-friendly cosmetic powders as alternatives to microplastics

In response to increasing regulatory constraints and growing environmental awareness, both the cosmetic industry and academic research are actively searching for alternative materials capable of replicating or surpassing the functional performance of microplastics while reducing their environmental impact [68]. Numerous initiatives have been initiated to mitigate the negative effects of microplastics, especially those that result from personal care and cosmetic products on marine and terrestrial ecosystems. These efforts focus on the development of biodegradable, bio-based, or naturally derived powders, as well as on advanced material engineering approaches aimed at tailoring particle morphology, surface characteristics, and functional performance [69]. In parallel, increasing consumer awareness regarding the environmental responsibility of synthetic polymers is accelerating the development and optimization of biopolymers derived from renewable natural sources. The increasing level of consumer awareness has resulted in a preference for formulations that utilize renewable resources and, where possible, natural ingredients [70]. Owing to their eco-friendly profile, safety, and biocompatibility, natural polymers are of relevance for cosmetic applications [71]. Materials such as starch, cellulose, alginate, chitosan, lignin, pectin, xanthan gum, agar, hyaluronic acid, guar gum, gelatin, collagen, and keratin can be engineered into microparticulate forms [72], [73] effectively incorporated into cosmetic formulations [74]. These materials exhibit broad applicability in skincare, haircare, and decorative cosmetics, where they function as stabilizing agents and rheological or sensory modifiers. Furthermore, chemical or physical modification of natural polymers through the incorporation of small molecules or synthetic moieties enables the development of semi-synthetic alternatives with tailored performance characteristics.

The transition away from conventional microplastics, therefore represents not only a regulatory challenge but also a scientific and technological opportunity to redesign cosmetic powders in line with sustainability, safety, and circular economy principles. Within this context, the identification, characterization, and performance evaluation of sustainable alternatives to microplastics (**Figure 1.4.1**) [14] in cosmetic powders emerge as a key research focus, providing the foundation for next-generation cosmetic formulations that balance high functional efficacy while minimizing environmental impact.

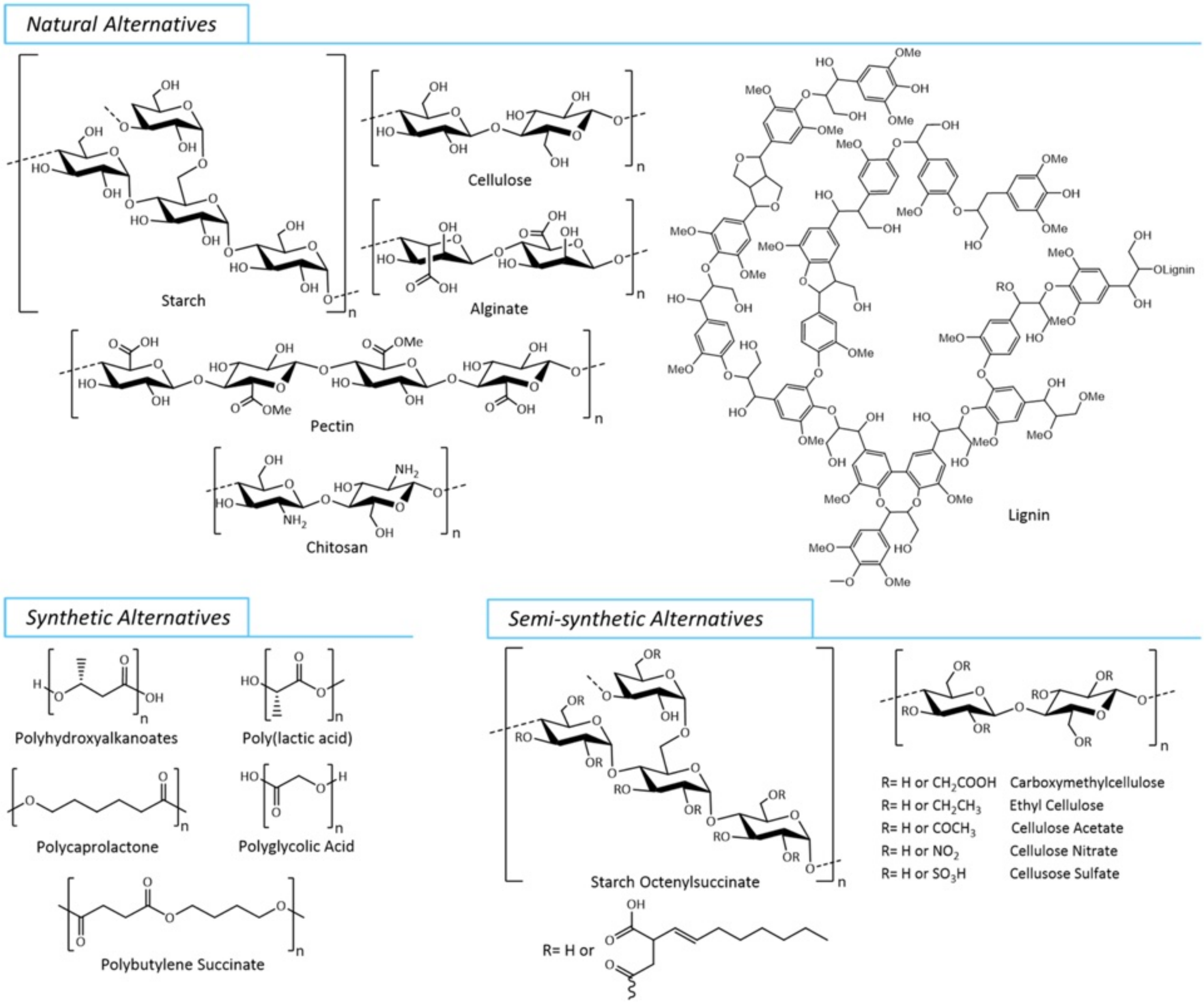

Figure 1.4.1 Main natural, semi-synthetic and synthetic alternatives employed to replace microplastics in cosmetic products [14].

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Aim

The present PhD research project is framed within the context of the MUSA (Multilayered Urban Sustainability Action) project, a strategic initiative promoted by the city of Milan aimed at promoting a sustainable transition at several levels of the city of Milan. In particular, this work is aligned with Spoke 5 of the MUSA project, which focuses on Sustainable Fashion, Luxury, and Design, and specifically addresses sustainability challenges within the cosmetics industry. Within this framework, the thesis investigates the development of environmentally friendly cosmetic formulations through the replacement of conventional synthetic microplastics with natural, biodegradable alternatives derived from renewable resources. This project aims to identify, design, modify, and apply natural polymer-based materials as sustainable substitutes for microplastics commonly used in cosmetic formulations, while maintaining or enhancing the functional performance, sensory properties, and stability of the final products. This research is strongly motivated by the increasing environmental and health concerns associated with microplastic pollution, as well as by the rapidly evolving regulatory landscape that is progressively restricting or banning the use of primary microplastics in cosmetic products, particularly in rinse-off formulations. In this context, the cosmetics industry faces an urgent need to develop effective and scalable alternatives that comply with regulatory requirements and meet consumer expectations for eco-safe products. To address this challenge, the research focuses on carbohydrate-based polymers, which represent a highly promising class of materials due to their biodegradability, renewability, abundance, and structural versatility. Specifically, tapioca starch, chitosan, and carboxymethyl cellulose (CMC) were selected as model natural polymers and systematically investigated as potential microplastic substitutes. These materials were processed into microparticles with controlled size, morphology, and surface characteristics using particle production techniques such as precipitation and spray drying. The production method played a crucial role in influencing the physicochemical and morphological properties of the resulting particles, as these parameters are critical for their functional performance in cosmetic systems. In addition, surface modification techniques were explored to alter the interfacial and rheological characteristics of the starting materials and enhance their compatibility with cosmetic formulations. The incorporation of hydrophobic molecules was investigated as a means to modulate surface properties and improve particle matrix interactions. The resulting microparticles were extensively characterized in terms of particle size distribution, morphology, and texture properties.

The second part of the project was focused on the formulation study. A base cosmetic emulsion was designed and optimized as a representative model system to evaluate the performance of the synthesized microparticles. The stability, textural properties, and rheological behavior of the base formulation were first assessed, followed by the incorporation of the natural microparticles as structuring agents, texture modifiers, and co-stabilizers. Particular emphasis was placed on understanding how particle composition, surface modification, and concentration influence emulsion stability, droplet size distribution, flow behavior, and sensory relevant properties. The results provided evidence that the synthesized microparticles could serve effectively and safely as co-stabilizers and structuring-modifying agents for sustainable cosmetic emulsion formulation, confirming the appropriateness of the proposed biodegradable starch microparticles. In contrast, chitosan and cellulose based microparticles did not yield satisfactory texture or stabilization under the tested conditions, pointing toward additional optimization and investigation.

This PhD project aims to provide a comprehensive and systematic approach to the replacement of microplastics in cosmetic formulations by combining material science, particle engineering, and formulation technology. By demonstrating the feasibility and limitations of starch, chitosan, and cellulose-based microparticles in cosmetic emulsions, the research contributes to the development of next-generation, microplastic-free cosmetic products. The outcomes of this work are expected to support the sustainable innovation of cosmetic formulations, in line with regulatory demands and the broader objectives of environmental protection and urban sustainability promoted by the MUSA project.

Project 1:

SEARCH OF NATURAL ALTERNATIVES FOR REPLACING MICROPLASTICS IN THE DEVELOPMENT OF COSMETIC FORMULATIONS

Chapter 1: Polysaccharide-based materials for sustainable cosmetic applications

1. Background

1.1. Natural biopolymers for sustainable application

The ongoing transition of the cosmetic industry toward “green cosmetics”, driven by the current international regulations that have banned or restricted the use of numerous chemical ingredients, together with the growing consumer demand for products with improved environmental sustainability, has led to extensive research to identify suitable alternatives to conventional ingredients derived from petrochemical sources [1]. As consumer awareness safer, more natural, and environmentally sustainable cosmetic products. Within this evolving landscape, biopolymers have emerged as a highly promising class of materials capable of addressing these challenges [2]. Polymers are a crucial raw material class for cosmetic formulations, essential in the production of high-performance products. The classification includes natural polymers (plant or animal origins), semi-synthetic polymers (chemically modified natural polymers, from plant or animal origins), and synthetic polymers (fermentation or chemical routes) that are composed of multiple repeating units (monomers), typically arranged in a chain [3].

Biopolymers are materials that are produced from renewable resources and have attracted increasing attention as sustainable alternatives to synthetic polymers across a wide range of industrial sectors, including cosmetics [4]. The characteristics of biopolymers can be modified by altering the chemical structure of the monosaccharide units and by changing the degree of polymerization. The structures of biopolymers vary, with linear or branched structures and different levels of complexity [5]. Their structural diversity enables them to perform a variety of functions in living organisms.

One of their most significant advantages over synthetic polymers is their inherent biodegradability and environmental compatibility, which substantially reduces long-term ecological impact [6].

In contrast to synthetic polymers, typically derived from petroleum-based sources and associated with prolonged environmental persistence, biopolymers can be efficiently degraded through biological processes into non-toxic by-products. In addition to biodegradability, the physicochemical and mechanical properties (flexibility, brittleness, and stiffness) of biopolymers should be comparable to those of conventional plastics [7]. Beyond their environmental benefits, biopolymers exhibit a variety of physicochemical properties that render them particularly attractive for cosmetic applications. Many biopolymers demonstrate excellent biocompatibility, a critical prerequisite for materials intended for direct and prolonged contact with the skin [8].

Furthermore, their ability to form hydrogels, films, and emulsions enables a high degree of formulation versatility in cosmetic products. Biopolymers also display remarkable water retention capacity, making them especially suitable for applications related to skin hydration and moisture management [9]. In addition, their tunable mechanical, rheological, and structural properties allow the design of cosmetic products with tailored characteristics, such as elasticity, adhesion, and texture, which are essential for consumer acceptance in skincare and beauty formulations [10]. Collectively, these features confer a competitive advantage over synthetic polymers, which often require the incorporation of stabilizers, preservatives, or additional chemical agents to achieve comparable performance.

Currently, polymers used in the cosmetic industry can be broadly classified into four main categories (i) synthetic polymers, (ii) polysaccharide-based polymers, (iii) protein-based polymers, and (iv) silicones [10] (**Figure 1.1.1**) [11] .These polymer classes perform distinct and often complementary roles within cosmetic formulations. In this context, polysaccharides have attracted considerable interest as potential candidates capable of fulfilling many of the functional roles traditionally associated with synthetic polymers and silicones.

Figure 1.1.1 The classification of polymers commonly used in cosmetics and cosmetic products [11].

1.2. Polysaccharide-based polymers

Polysaccharides, which belong to the third main class of biopolymers, are complex carbohydrates characterized by the presence of multiple hydroxyl groups along their backbone, a structural feature that has enabled their exploitation in cosmetic and personal care formulations for centuries [11]. They are categorised according to their chemical structure, which consists of monosaccharide units joined by glycosidic bonds [12].They can also form covalent bonds with other structures, such as lipids, peptides, and amino acids. In contrast to heteropolysaccharides, which are heteroglycans that have multiple monosaccharides, homopolysaccharides are homoglycans that are made up of the same monosaccharide [13].

Polysaccharides can also be categorized based on their functional roles in organisms. Storage polysaccharides and structural polysaccharides are the two main categories. Storage polysaccharides are predominantly responsible for storing energy as food supplies. Examples of storage polysaccharides are mainly glycogen and starch [14]. Structural polysaccharides are responsible for providing support and structure to cells and tissues, leading to the formation of various structural components of organisms. Examples of structural polysaccharides, among others, comprise cell wall components such as cellulose, hemicellulose, and lignin [15].

Their long-standing use is primarily attributed to their favorable biological and physicochemical properties, including biodegradability, biocompatibility, non-toxicity, renewability, and wide availability [16]. Moreover, polysaccharides generally exhibit a superior safety profile compared to synthetic polymers, thereby reducing potential risks to both human health and the environment associated with their use. A wide variety of polysaccharides is available, differing in terms of charge (cationic, anionic, non-ionic, or amphoteric) and molecular conformation (typically rigid helices or random coils) [17],[18]. Their properties can be modified by temperature, concentration, and the presence of salts. This structural and chemical diversity provides formulators with a broad spectrum of functional properties, enabling polysaccharides to be employed in multiple cosmetic applications, such as rheology modifiers, conditioners, suspending and healing agents, moisturizers, hydrating agents, and emulsifiers [19].

Based on these considerations, three polysaccharides (starch, chitosan, and cellulose derivative) were selected in this study as model systems, owing to their favorable safety profile, wide availability, and tunable physicochemical properties, which make them particularly suitable for cosmetic and formulation applications.

1.3. Starch

Starch, the second most abundant natural biopolymer after cellulose, is a plant storage polysaccharide, composed of a mixture of linear poly(1,4-α-d-glucopyranose) (amylose) and branched poly(1,4-α-d-glucopyranose) with branches of (1,6-α-d-glucopyranose) (amylopectin) (**Figure 1.3.1**)

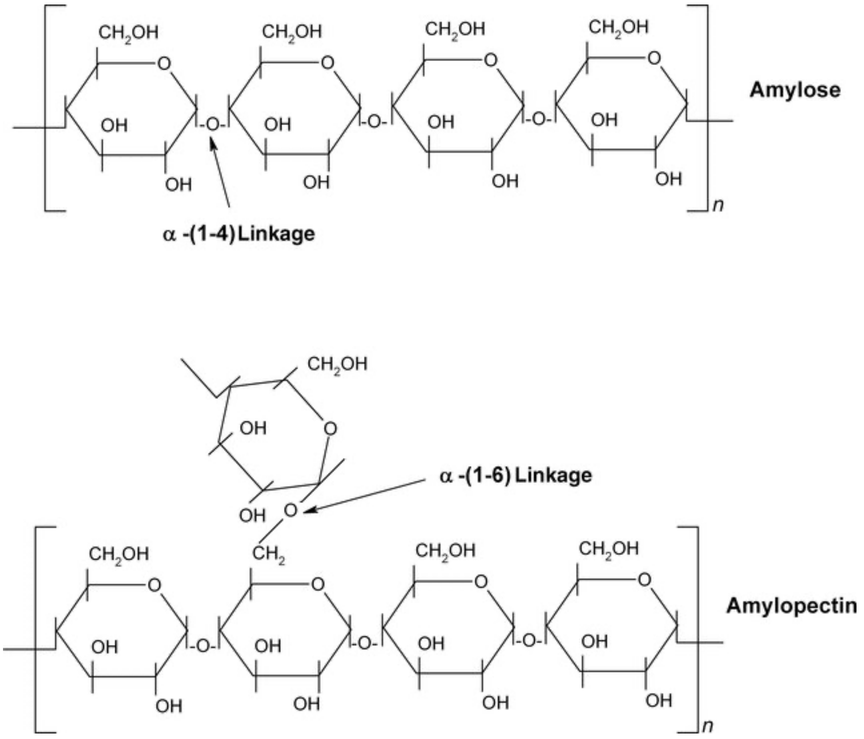

Figure 1.3.1 Chemical structure of starch with amylose and amylopectin units [62].

The quantity and composition of the two polysaccharides can vary depending on their botanical origins and harvested time, and they are commonly accumulated in macroscopically dense granules [7]. Branch chains of amylopectin form double helices and contribute to the crystalline structure of the granules, while amylose is amorphous and interspersed among amylopectin molecules [20]. The combination of amorphous and crystalline produces three main types of semi-crystalline starch granules, which are classified as type A (found in cereals) [21], type B (common in tubercles, cladodes, and fruits) [22] , and type C (generally found in legumes) [23]. While amylose mainly presents a linear structure enabling it to form gels and strong films, amylopectin displays a mainly branched conformation. This structural feature affects its crystallinity and physicochemical properties. Such structural differences define the functionality of starch in cosmetic applications: film formation, viscosity control, and moisture retention [24].

An important process in starch-based applications is gelatinization, which occurs when starch granules undergo structural disruption upon heating above a characteristic temperature, typically in the range of 60–70 °C. This process involves the progressive weakening of intermolecular hydrogen bonds within the starch granule and the formation of new hydrogen bonds between the hydroxyl groups of starch polymers and surrounding water molecules. At sufficiently high water contents, water acts as a plasticizer for the amorphous regions of the starch granules, promoting granule swelling, partial solubilization, and loss of native crystalline order.

Another process that must be considered in industrial starch applications is retrogradation, in which the starch molecule recovers parts of its crystalline structure. During retrogradation, starch polymers, particularly amylose and, to a lesser extent, amylopectin, tend to realign into more ordered structures, leading to changes in texture, opacity, and mechanical properties [25].

In its native form, starch exhibits several inherent limitations that restrict its direct applicability due to the high water solubility and swelling power, low gelatinization temperature and tendency to suffer retrogradation, in addition to having low tensile strength [26]. To overcome these constraints and broaden its functional utility, starch is commonly subjected to modification strategies aimed at enhancing its physicochemical performance. The functionality of native starch can be tailored through different modification approaches, enabling its use in diverse industrial applications. These approaches include physical, chemical, enzymatic, and biotechnological or genetic methods. Among them, physical and chemical modification techniques represent the most widely adopted strategies [27]. In physical modification processes, the chemical structure of the D-glucopyranosyl units within the starch backbone remains unaltered, and the modifications primarily affect the supramolecular organization and physicochemical behavior of the starch granules. In contrast, chemical modification involves the deliberate introduction of functional groups into the starch structure, thereby altering its molecular architecture and interfacial properties [28]. Common chemical modification methods include oxidation, acid hydrolysis, acetylation, and cross-linking, all of which lead to the incorporation of new functional moieties into the starch granules.

As a result of these modification processes, several key properties of starch, such as moisture affinity, solubility, thermal and storage stability, viscosity, and emulsifying capacity, can be significantly altered. The extent and nature of these property changes depend on both the type of substituent introduced and the degree of substitution, which together determine the final functional profile of the modified starch [29]. Physically, chemically, or enzymatically modified starches, like pregelatinized, oxidized, cationic, and esterified, are generally used in cream, lotion, and emulsion applications. The use of modified starch generally creates improvement in dispersibility, stability, and tactility while imparting moisturizing, softening, and absorbent properties [30].

1.4. Chitosan

Chitosan is the deacetylated derivative of chitin, a naturally occurring polysaccharide that can be found in a broad range of living organisms. Chitin is the main component of the exoskeleton of many invertebrates (crustaceans, mollusks, and insects) but it also serves as a structural component in the

cell wall of different fungi and yeasts [31]. Chitosan is composed of randomly distributed β-(1–4) - linked d-glucosamine and N-acetyl-d-glucosamine with a repeated sequence (**Figure 1.4.1**).
Its structure is characterized by primary hydroxyl groups, secondary hydroxyl groups, and amino functional groups, which provide solubility and hydrophilicity to chitosan.

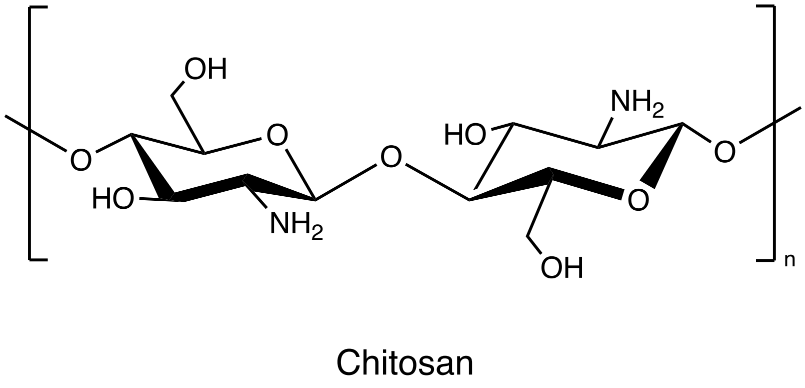

Figure 1.4.1 Schematic representation of the chemical structure of chitosan

Chitosan is a colourless, nitrogenous, natural cationic gum that becomes viscous and concentrated when neutralized with acid, making it a versatile material that can be utilized in gels, powders, membranes, fibers, colloidal films, and beads. Chitosan is characterized by biocompatibility and non-toxicity, making it valuable for food, pharmaceutical, biomedical, and cosmetic industries. This characteristic makes it an ideal biopolymer for cosmetic applications, such as creams and lotions [32]. Chitosan is widely recognized for its filmogenic, moisturizing, antimicrobial, and biocompatible characteristics [33], [34]. Chitosan is applied in skin, hair, and oral care, formulation of cosmetics and personal care products as a humectant and conditioning agent due to its ability to interact electrostatically with negatively charged biological surfaces. This interaction improves skin hydration, reduces trans-epidermal water loss, and creates a protective film as barrier [34]. In addition to these recognized roles, chitosan exhibits a pronounced ability to bind water, thereby contributing to skin hydration, and can be effectively employed as a thickening agent, rheology modifier, and emulsion stabilizer [35]. Furthermore, chitosan possesses antioxidant, antimicrobial, and wound-healing properties, which extend its applications in dermatological and cosmeceutical preparations. This versatile biopolymer can be formulated in every type of cosmetics, including emulsions, gels, foams, sticks, and aerosols [36]. Chitosan is soluble in acidic aqueous solutions and can easily produce different conformations such as micro, nano, and milliparticles, films, scaffolds, and fibers, among others [37].

1.5. Sodium carboxymethyl cellulose

Sodium carboxymethyl cellulose (CMC) is the most widely produced and industrially utilized cellulose derivative. It is a linear, non-toxic, anionic, water-soluble anionic polysaccharide, derived from the partially substituted ionic carboxymethyl (-CH_2COOH) of the cellulose hydroxyl group [38]. It is typically synthesized via a Williamson etherification reaction, which involves an initial alkalization step in which cellulose is treated with sodium hydroxide to form sodium cellulosate. Subsequently, sodium chloroacetate is generated by dissolving chloroacetic acid in sodium hydroxide, and the chlorine atom of sodium chloroacetate reacts with sodium cellulosate to yield carboxymethyl cellulose [39]. Structurally, CMC is a copolymer composed of two repeating units—β-D-glucose and β-D-glucopyranose 2-O-(carboxymethyl) monosodium salt—linked through β-1,4-glycosidic bonds (**Figure 1.5.1**). The introduction of carboxymethyl substituents (–CH_2–COOH) onto the glucose residues of the cellulose backbone significantly enhances the polymer's interfacial and solubility characteristics. As a result, CMC exhibits a broad spectrum of functional properties, including thickening, suspending, binding, film-forming, gelling, stabilizing, and water-retention capabilities, as well as protective colloid behavior and rheological control [40]. The polymer is characterized by its hydrophilic, bioadhesive, pH-sensitive, non-toxic, and gelling properties [41].

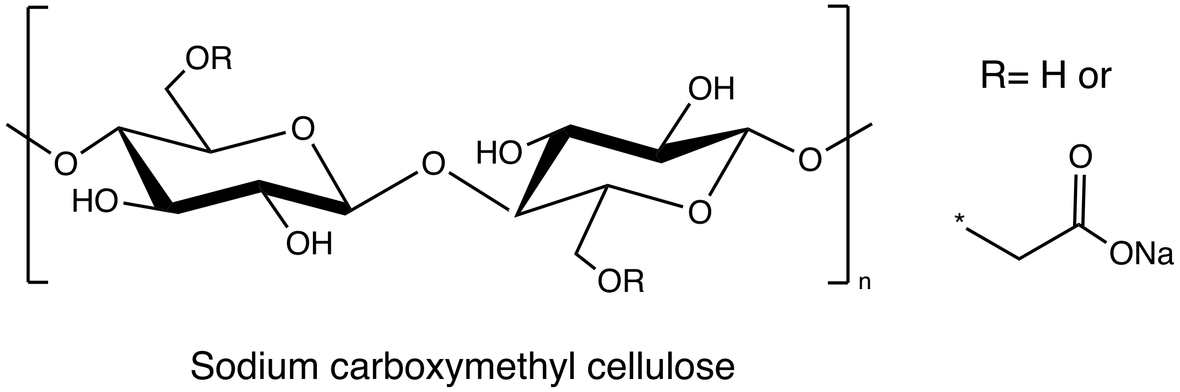

Figure 1.5.1 Schematic representation of the chemical structure of Sodium carboxymethyl cellulose

CMC, as thickening agent, plays a key role in cosmetic and medical formulations by enhancing product consistency, stability, and overall performance, thereby making it a highly versatile and valuable functional ingredient. In addition, CMC exhibits notable resistance to a range of solvents, including water, which further contributes to its reliability and robustness in diverse formulation environments. The high molecular weight of cellulose derivatives such as CMC enables effective moisture binding and retention, a property that underlines their widespread use in skincare products aimed at improving skin hydration and elasticity [42].

Moreover, cellulose derivatives exhibit a good compatibility with a wide range of active cosmetic ingredients, which not only expands their functional role but also promotes the uniform dispersion of bioactive compounds within gel-based systems. This homogeneous distribution is critical for achieving consistent dosing and optimizing the overall performance and efficacy of cosmetic formulations. Consequently, CMC and related cellulose derivatives are widely recognized in the cosmetic field for their hydrating and stabilizing functions, as well as for their capacity to enhance sensory properties and ensure long-term formulation stability. Taken together, the multifunctional nature of polysaccharides, as exemplified by cellulose derivatives such as CMC, underscores their importance in the rational design of cosmetic gels. In this context, they contribute to improved hydration, more efficient delivery of active cosmetic ingredients, and increased formulation stability.

1.6. Biopolymer-based microparticles

The use of biopolymers for the fabrication of MPs has attracted considerable interest as a strategy to mitigate the health concerns commonly associated with synthetic polymers, including chronic inflammatory and immunological responses as well as in vivo toxicity [43]. In parallel, biopolymer-based MPs represent a sustainable alternative to non-degradable, petrochemical-derived microplastics, which are known to persist in the environment and accumulate along the food chain. Beyond their traditional role as inert exfoliating beads, such microparticles can be engineered to perform active functional roles within cosmetic formulations, including rheology modification, emulsion stabilization, texture enhancement, and controlled release of active ingredients [44]. Moreover, the high density of functional groups in biopolymers, such as hydroxyl, amine, and carboxylic acid groups, provides versatile sites for chemical modification, enabling the introduction of tailored bioactivities into the resulting MPs. One of the most promising approaches for the cosmetic exploitation of natural polymers is their processing into microparticles, as this strategy enables fine control over particle size, morphology, and surface properties, thereby facilitating their integration into complex cosmetic formulations. MPs are particles of characteristic sizes of 1–1000 µm [45]. Selecting the proper process is crucial for producing polymeric microparticles, as it directly influences particle size, morphology, surface characteristics, and ultimately their functional performance within the final formulation. Available approaches include physical methods (such as spray drying, extrusion, spray coating, and precipitation) and chemical methods (such as interfacial polymerization and molecular inclusion) [46]. To properly select the process for producing microparticles, several fundamental aspects must be considered, including the specific final application, the target particle size and the desired particle morphology, such as a spherical shape to mimic conventional microplastics. Additional factors include the physicochemical properties of the polymer, the degree and type of chemical modification required, the intended functional performance within the formulation (e.g., emulsifying, thickening, or structuring effects), as well as practical considerations related to process scalability, reproducibility, and overall production cost [46] .

Nevertheless, achieving precise control over the size and morphology of biopolymer-based MPs remains challenging, primarily due to batch-to-batch variability in the raw biopolymers, which can limit reproducibility and hinder their broader technological application [45].

In parallel, comprehensive characterization of microparticles is essential to establish structure–property relationships. Techniques such as scanning electron microscopy (SEM) provide insight into particle morphology and surface structure, while spectroscopic methods (e.g., FT-IR) enable the identification of chemical modifications. Particle size distribution analysis and rheological measurements further contribute to understanding dispersion behavior and functional performance within complex formulations.

2. Results

Based on the concerns described above, the experimental results presented in this chapter aim to demonstrate how natural polymers can be engineered into functional microparticles suitable for cosmetic applications. In particular, the results section focuses on the chemical modification, particle development, and functional characterization of selected polysaccharides with the aim of tailoring their surface properties, morphology, and performance within representative cosmetic systems. Among the biopolymers, starch, chitosan and CMC were selected due to their wide availability, low cost, biodegradability, and well-established industrial relevance, as well as the high density of hydroxyl groups along their backbone, which enables versatile chemical functionalization.

2.1. Preparation of starch MPs: Synthesis and characterization

The physicochemical properties of starch, including those of its main components amylose and amylopectin, can be tailored through physical and chemical processing to adapt to specific applications. To produce starch-based microparticles, a preliminary optimization study was conducted by systematically varying both the concentration of starch and the processing temperature in order to identify suitable conditions for particle formation. Different starch concentrations and thermal treatments were initially evaluated, which ultimately led to the definition of a reproducible protocol for microparticle production.

Tapioca starch (50 g L^{-1}) was dispersed in deionized water and maintained at 50 °C under continuous stirring for approximately 2 h to promote hydration and partial gelatinization of the starch granules, yielding a homogeneous aqueous dispersion. Subsequently, starch microparticles were produced by the precipitation (antisolvent) technique (**Figure 2.1.1**). In this method, microparticles are prepared by the miscibility of two solvents (solvent-antisolvent) under controlled stirring conditions [47].

The starch is completely dissolved in the solvent (water), and then the solution is added to the antisolvent. After, the organic solvent was removed through vacuum filtration with a filter membrane of 0.2 µm.

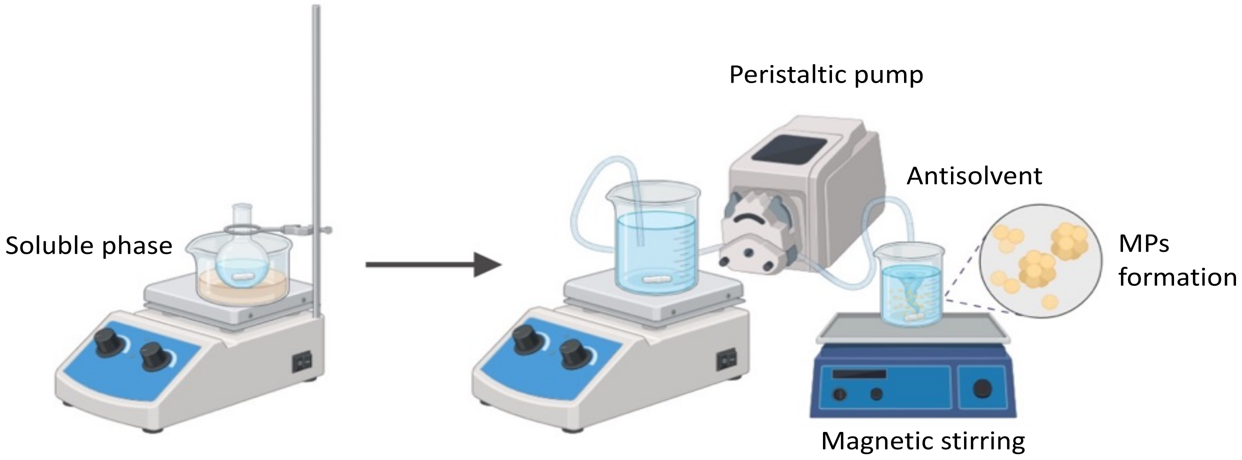

Figure 2.1.1 Schematic representation of the microparticle manufacturing method used in this study: precipitation process based on solvent- non solvent mixing.

Both ethanol and isopropanol were evaluated as antisolvents for the precipitation of starch microparticles. In this process, microparticle formation is driven by a rapid reduction in starch solubility caused by the change in solvent polarity and the local supersaturation generated upon mixing the solvent and the antisolvent [48]. This supersaturation promotes nucleation and growth of solid starch domains, ultimately leading to the formation of discrete microparticles. The physicochemical properties of the resulting particles, including their morphology and powder texture, were found to be strongly dependent on both the nature of the antisolvent and its volumetric fraction in the solvent mixture.

When absolute ethanol was employed, starch precipitation resulted predominantly in the formation of irregular aggregates, which can be attributed to an excessively rapid and non-equilibrated solvent exchange between the aqueous and organic phases; whereas, at low ethanol concentrations, no solid microparticles were obtained and the system remained in the form of colloidal dispersions. At intermediate ethanol concentrations (20% and 40% v/v), the formation of a milky dispersion was consistently observed, which was indicative of the generation of suspended starch microparticles (**Figure 2.1.2**) This concentration range was therefore identified as providing an optimal balance between solvent–antisolvent miscibility and controlled supersaturation, enabling reproducible microparticle formation. On this basis, ethanol concentrations between 20% and 40% v/v were selected as the most suitable conditions for the precipitation-based production of starch microparticles.

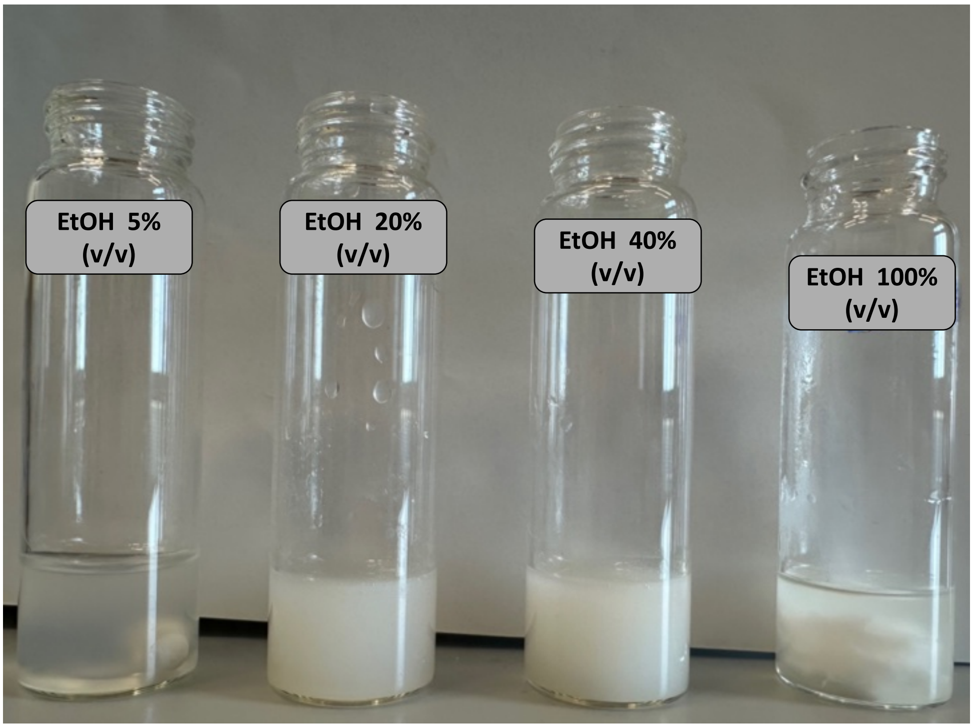

Figure 2.1.2 Photographs of starch dispersions prepared by precipitation in aqueous–ethanol systems at different ethanol concentrations (v/v), showing the macroscopic appearance of the resulting suspensions.

Isopropanol exhibited a qualitatively similar antisolvent behavior; however, due to its lower polarity and higher hydrophobicity compared to ethanol, it tended to promote a more rapid precipitation process, which increased the tendency toward particle aggregation and reduced morphological control [49]. Ethanol was selected as the preferred antisolvent for the further reactions.

Once the optimal protocol for starch microparticle production had been established in terms of starch concentration, processing temperature, and antisolvent fraction, attention was shifted toward improving the intrinsic functional properties of the resulting material. Although the precipitation process enabled the formation of starch-based microparticles with controlled size and morphology, native starch remains inherently hydrophilic due to the high density of hydroxyl groups along its backbone. This intrinsic hydrophilicity limits its compatibility with lipophilic phases, reduces its affinity for oil–water interfaces, and negatively affects its sensory performance when applied as a powder in cosmetic formulations, particularly in terms of flowability and skin feel [50], [51].

In order to enhance the hydrophobic character of the starch microparticles and to obtain a free-flowing powder with improved sensory and rheological attributes, a chemical modification strategy was therefore followed. The main objective of this approach was to introduce hydrophobic or amphiphilic moieties onto the starch backbone, thereby modulating its surface energy, wettability, and interfacial behavior. Different modifying agents were investigated, including oleic acid, poly (isobutylene-alt-maleic anhydride), and the amino acid tryptophan. These molecules were selected with the aim of imparting complementary functionalities, such as increased hydrophobicity and improved powder flow properties, while preserving the biodegradable and biocompatible nature of the starch matrix.

The first modification reaction investigated was the functionalization of tapioca starch with oleic acid (**Figure 2.1.3**), which was chosen as a model hydrophobic moiety due to its long aliphatic chain, widespread use in cosmetic formulations, and favorable safety and sustainability profile. Oleic acid is a monounsaturated fatty acid (cis-9-octadecenoic acid) characterized by an 18-carbon hydrocarbon chain bearing a single cis double bond and a terminal carboxylic acid group. This molecular structure confers a pronounced amphiphilic character, with a hydrophobic alkyl tail and a polar carboxyl functionality capable of participating in esterification reactions with the hydroxyl groups of starch.

From a physicochemical perspective, oleic acid is widely recognized for its emollient, lubricating, and skin-conditioning properties, as well as for its ability to reduce friction and improve sensory perception in topical formulations [52]. Its incorporation onto the starch backbone was therefore expected to decrease the overall hydrophilicity of the biopolymer, increase its affinity for lipophilic phases, and promote the formation of starch-based microparticles with enhanced flowability and greater suitability for cosmetic applications.

During the reaction, heating the starch dispersion in the presence of oleic acid at 50 °C facilitated the partial gelatinization of starch granules and promoted molecular interactions between amylose helices and the hydrophobic tail of oleic acid [53].

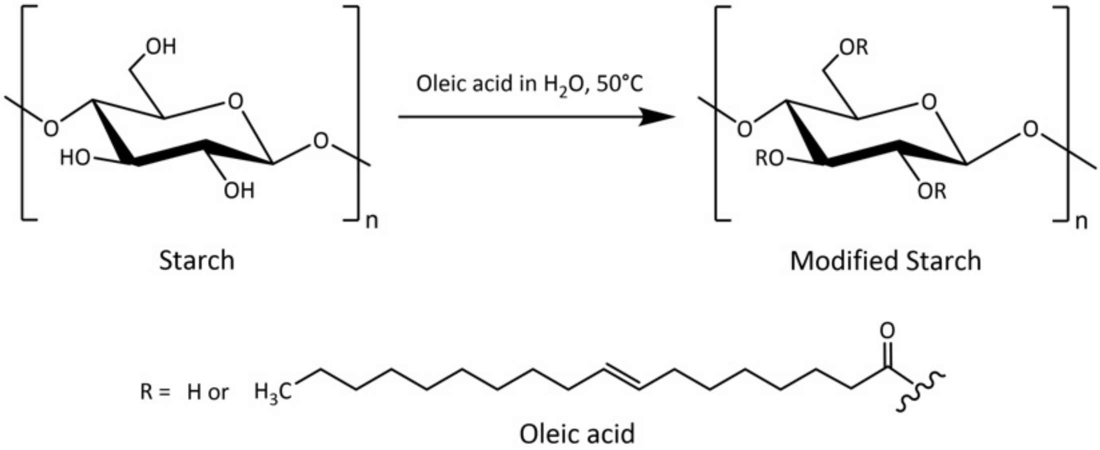

Figure 2.1.3 Proposed esterification reaction between hydroxyl groups of tapioca starch and oleic acid, resulting in hydrophobically modified starch.

The structural modification increases the overall hydrophobicity of the polymeric matrix, thereby improving the compatibility of the resulting microparticles with lipidic environments and enhancing their potential adhesion and interaction with the skin surface. The precipitation of the modified starch phase in a hydroalcoholic solution allowed the controlled formation of microparticles through phase separation, yielding well-defined and easily recoverable structures after lyophilization [54].
To investigate another alternative solvent approach for microparticle formation, the aqueous starch–oleic acid solution was precipitated into pure 1-octanol, a non-polar solvent immiscible with water. The immiscibility of 1-octanol with the aqueous phase allowed controlled formation of discrete microparticles through phase separation. Additionally, the use of octanol facilitated the recovery and reuse of the antisolvent, minimizing waste and enabling its recycling for subsequent syntheses. This approach provided an effective and sustainable strategy for producing starch-based microparticles with defined morphology and hydrophobic characteristics.

Fourier transform infrared spectroscopy (FT-IR) was employed to investigate the chemical structure and functional group composition of native and chemically modified starch samples. The FT-IR spectrometer simultaneously collects high-resolution information over a wide spectral range (between 4000 and 400 cm^{-1}). Spectra were collected using an attenuated total reflectance (ATR) accessory, which enables direct analysis of solid and semi-solid samples without extensive sample preparation. In the ATR configuration, the infrared beam undergoes total internal reflection within a high-refractive-index crystal, generating an evanescent wave that penetrates a few micrometers into the sample in contact with the crystal surface. The structural and morphological characterization of the tapioca starch microparticles provided further confirmation of the successful modification achieved through oleic acid incorporation. FT-IR spectroscopy was employed to evaluate the chemical interactions between starch and oleic acid within the microparticle matrix. The absorption band observed at 2900 cm^{-1}, attributed to the symmetric stretching vibration of methylene ($–CH_2–$) groups, together with the characteristic C=O stretching band at 1749 cm^{-1} corresponding to the carboxylic group of oleic acid, confirmed the occurrence of molecular interactions between the two components (**Figure 2.1.4**).

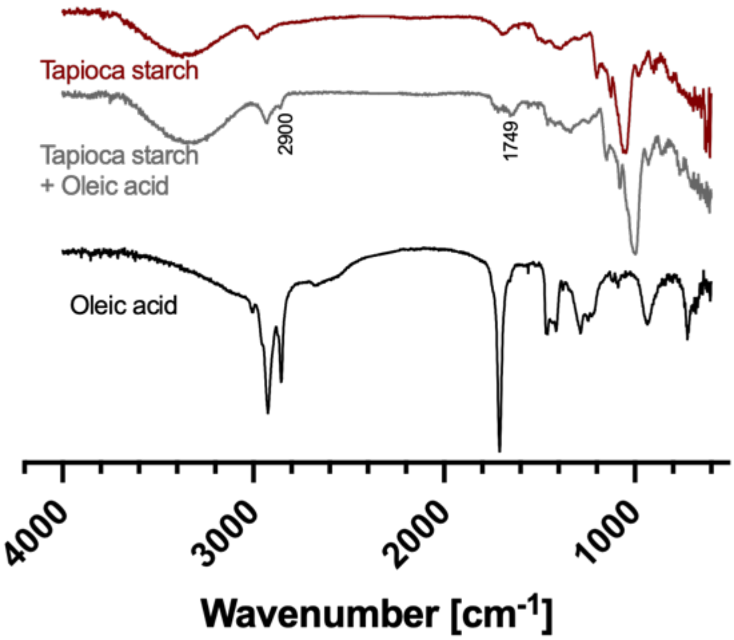

Figure 2.1.4 FT-IR spectra of tapioca starch, illustrating the main vibrational modes of the polymer matrix and the encapsulated compound.

The morphology of the obtained microparticles was analyzed using scanning electron microscopy (SEM). Both unmodified and oleic acid–modified starch microparticles exhibited a generally spherical morphology with diameters ranging from approximately 8 to 14 µm. The surface of the modified particles appeared slightly smoother and more compact compared to the native starch granules, suggesting a partial rearrangement of the polymeric chains upon complex formation. (**Figure 2.1.5**).

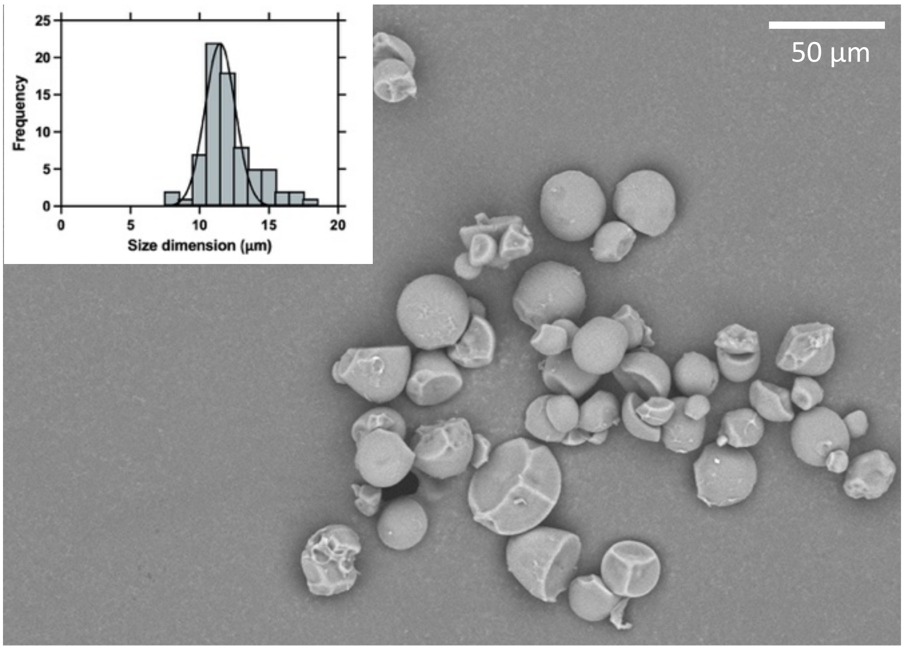

Figure 2.1.5 SEM image of oleic acid starch microparticles.

Despite the morphological similarity, the incorporation of oleic acid was found to alter the rheological behavior of the starch-based material, indicating modifications in interparticle interactions and overall texture. These changes are particularly relevant in cosmetic formulations, as they may influence product stability, spreadability, and sensory performance.

The microparticles synthesized in the octanol medium exhibited comparable dimensions but showed an increased surface density and reduced hydrophilicity, likely due to the higher degree of lipid association promoted by the non-polar solvent environment. Such features highlight the potential of solvent polarity and lipid content as key parameters to fine-tune the physicochemical and functional properties of starch-derived microparticles for cosmetic and dermatological applications.

Following the optimization of the precipitation protocol to produce native tapioca starch microparticles, a second functionalization strategy was explored using poly (isobutylene-alt-maleic anhydride) (**Figure 2.1.6**). The choice of this polymer was driven by several considerations. Poly (isobutylene-alt-maleic anhydride) is commercially available, cost-effective, and widely regarded as biocompatible, which makes it attractive for cosmetic use [55].

From a chemical point of view, poly (isobutylene-alt-maleic anhydride) is composed of repeating succinic anhydride units alternating with isobutylene moieties. The presence of cyclic anhydride functionalities offers highly reactive sites that can undergo nucleophilic ring-opening reactions with the hydroxyl groups abundantly present on the starch backbone. This feature enables the formation of ester linkages between starch and the polymer, resulting in a grafted structure with increased hydrophobic character imparted by the polyisobutylene segments.

Such a modification was expected to enhance the flowability, water resistance, and overall cosmetic sensorial performance of the resulting starch-based microparticles.

The functionalization of starch with poly (isobutylene-alt-maleic anhydride) was carried out following the same general protocol previously optimized for oleic acid modification, with appropriate adjustments in reagent ratios. Briefly, tapioca starch was dispersed in water and heated at 50°C under stirring to promote partial gelatinization and increase the accessibility of hydroxyl groups. The poly (isobutylene-alt-maleic anhydride) was then introduced into the reaction medium, allowing the anhydride groups to react with starch through esterification. After completion of the reaction, the modified starch was processed into microparticles using the antisolvent precipitation technique.

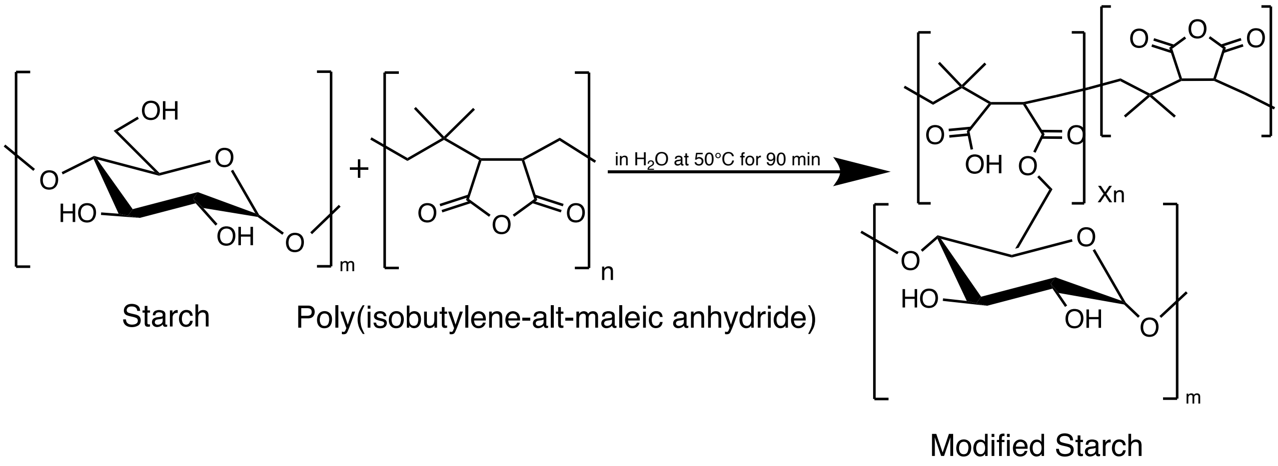

Figure 2.1.6 Schematic representation of the reaction between tapioca starch and Poly (isobutylene-alt-maleic anhydride).

FT-IR spectroscopy of the resulting reaction is shown in **Figure 2.1.7**. The bands detected at 2920–2850 cm^{-1} correspond to C–H stretching vibrations of aliphatic groups, arising from both the starch backbone and the isobutylene units of poly (isobutylene-alt-maleic anhydride) confirming the presence of the synthetic polymer within the system. In addition, a distinct absorption band observed at 1716 cm^{-1} can be attributed to the C=O stretching vibration of carboxylic groups, formed as a consequence of the opening of the maleic anhydride ring following its interaction with the hydroxyl groups of starch.

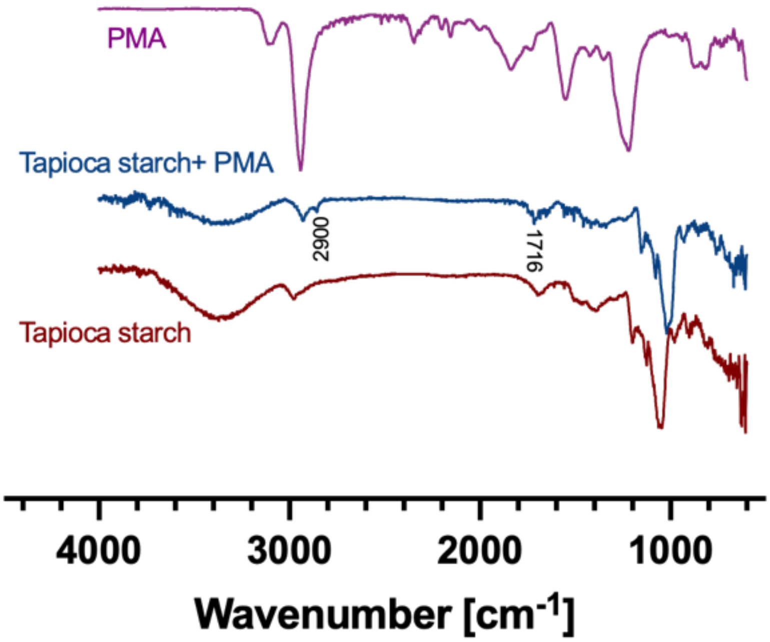

Figure 2.1.7 FT-IR spectra of tapioca starch PMA, illustrating the main vibrational modes of the polymer matrix

Although the starch–poly (isobutylene-alt-maleic anhydride) system allowed the formation of microparticles, the obtained powder showed poor flow behavior and a slightly cohesive texture upon handling, resulting in unsatisfactory rheological and sensorial characteristics.

In parallel, both oleic-acid-modified starch and poly (isobutylene-alt-maleic anhydride) -modified starch were also processed using spray drying as an alternative particle formation method. In this approach, starch was first fully solubilized at 90 °C to ensure a homogeneous feed solution. The resulting dispersion was then atomized into a stream of hot air inside a spray dryer, where rapid solvent evaporation led to the formation of dry microparticles. Spray drying is a widely employed industrial technique to convert liquid feeds into dry powders [56]. Since both aqueous and organic solvents can be processed, this method is particularly suitable for combining materials with diverse physicochemical characteristics in order to enhance their functional performance. One of the main advantages of spray drying lies in the ability to tailor particle properties, such as size, morphology, surface texture, and surface composition, through the optimization of formulation parameters and process conditions (**Figure 2.1.8**). The spray drying process can be divided into three fundamental stages: atomization of the liquid feed, solvent evaporation, and collection of the dried particles [57]. Atomization involves the conversion of a liquid into fine droplets and can be achieved using different types of atomizers.

Once atomized, the droplets enter a drying chamber through which a stream of hot drying gas flows. Separation of the dried particles from the exhaust gas is most achieved using centrifugal cyclone separators. In these systems, the gas enters the cyclone tangentially at the top, generating a spiral motion. Under the action of centrifugal forces, solid particles are driven toward the cyclone walls and subsequently collected at the bottom, while the cleaned exhaust gas is filtered and discharged. Despite their widespread use, cyclones present certain limitations, as part of the product may adhere to the walls of the drying chamber or cyclone, leading to material losses and reduced process efficiency [58].

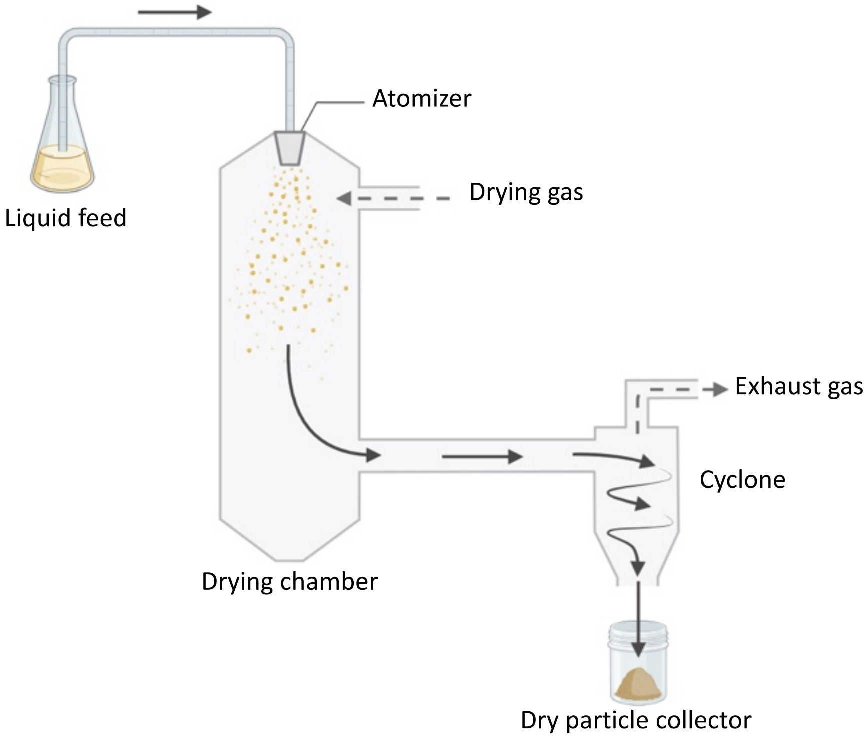

Figure 2.1.8 Schematic representation of the microparticle manufacturing method used in this study: spray-drying process involving atomization of the polymer solution and instantaneous solvent evaporaton to obtain dry microparticles

However, morphological analysis of the spray-dried oleic-acid-modified starch particles revealed the formation of hollow structures, likely due to rapid water evaporation and shell formation at the droplet surface during drying (**Figure 2.1.9**). In addition to the unfavorable particle morphology, a relatively low powder recovery yield was observed, attributable to product losses on the chamber walls and cyclone separator. These limitations significantly reduced the practical suitability of spray drying for the production of functionalized starch microparticles within the scope of this work.

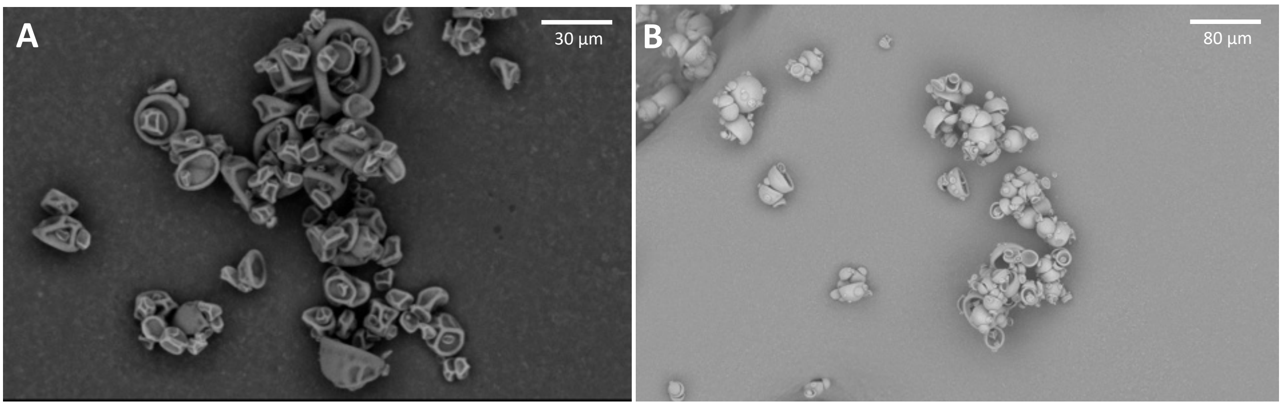

Figure 2.1.9 SEM photographs of spraydryed microparticles: **A**) Starch oleic microparticles; **B**) Starch Poly (isobutylene-alt-maleic-anhydride) microparticles.

For these reasons, the antisolvent precipitation method was ultimately preferred for both oleic-acid- and poly (isobutylene-alt-maleic anhydride) modified starch systems. Compared to spray drying, precipitation afforded higher powder yields, better control over particle morphology, and more compact, solid microparticles, which are more suitable for cosmetic applications requiring smooth texture, improved flowability, and enhanced sensory performance.

Following the functionalization of tapioca starch with oleic acid and poly (isobutylene-alt-maleic anhydride), a further modification strategy based on the aminoacid tryptophan was investigated. Tryptophan was selected for a dual rationale. From a structural perspective, the presence of an indole aromatic ring confers a pronounced hydrophobic character [59], which is expected to enhance the interfacial properties of starch-based microparticles and improve their sensory performance when applied to the skin. In addition, tryptophan exhibits a characteristic absorption maximum around 280 nm [60], falling within the UVC region and partially extending into the UVB range. This intrinsic optical property makes tryptophan an attractive functional moiety for the development of bio-based powders with potential UV-absorbing capability, opening perspectives for their use as multifunctional cosmetic ingredients, including sun-care applications.

To exploit these features, a two-step functionalization approach was adopted. In the first step, tryptophan was covalently linked to poly (isobutylene-alt-maleic anhydride) to obtain a modified polymer (PMW), which acted as a reactive and amphiphilic intermediate. Poly (isobutylene-alt-maleic anhydride) was reacted with tryptophan at 60 °C under continuous stirring for 24 h, conditions that promote the nucleophilic opening of the anhydride rings without causing polymer degradation. The reaction is driven by the nucleophilic attack of the primary amino group of tryptophan on the electrophilic carbonyl carbon of the maleic anhydride unit. This attack results in the opening of the cyclic anhydride and the formation of an amide linkage, accompanied by the generation of a neighboring carboxylic acid group. Poly (isobutylene-alt-maleic anhydride) was chosen due to its established biocompatibility, commercial availability, and high reactivity, arising from the presence of repeating succinic anhydride units along the polymer backbone [52]. These anhydride groups enable efficient coupling with nucleophilic functionalities, such as the amino group of tryptophan, while the polyisobutylene segments contribute additional hydrophobic character (**Figure 2.1.10**).

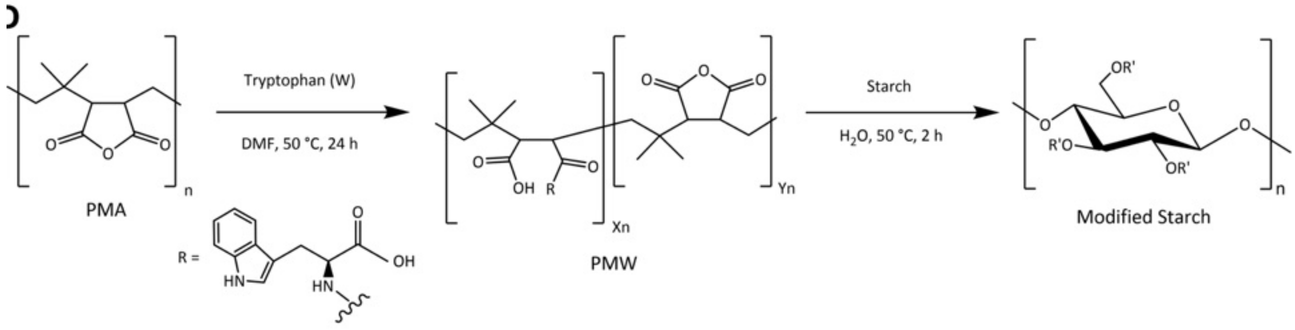

Figure **2.1.10** Proposed mechanism for the functionalization of starch with tryptophan (W).

The resulting polymer (named PMW) was subsequently reacted with tapioca starch under conditions analogous to those previously optimized for oleic acid functionalization. This strategy enabled the transfer of the hydrophobic and functional features of the amino-acid-modified polymer onto the starch backbone, ultimately leading to the formation of functionalized starch microparticles via antisolvent precipitation.

FT-IR spectroscopy provided clear evidence of successful chemical modification. In the spectra of PMW, the band at 1654 cm^{-1} is consistent with the formation of amide bonds following the nucleophilic ring opening of maleic anhydride units by tryptophan (**Figure 2.1.11 A**) In the spectra of PMW-functionalized starch microparticles, the appearance of absorption bands in the 1700–1740 cm^{-1} region was observed, consistent with the presence of carbonyl functionalities introduced through the PMW moieties. Additionally, noticeable changes in the carbohydrate fingerprint region (1000–1150 cm^{-1}) were detected, indicating modifications within the glycosidic framework of starch. These spectral features agree with structural alterations commonly associated with Poly (isobutylene-alt-maleic anhydride)-derived functionalization and confirm that the chemical environment of the polysaccharide was effectively modified (**Figure 2.1.11 B**).

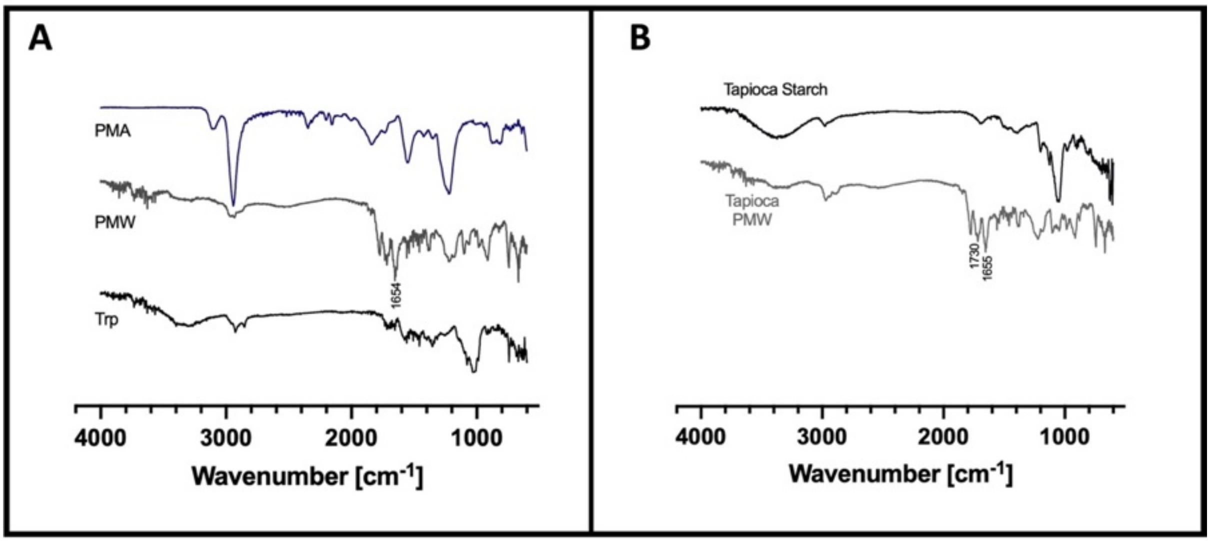

Figure 2.1.11 FT-IR spectra of PMW (A) and Tapioca starch PMW (B).

Morphological analysis by SEM revealed that the PMW-functionalized starch microparticles exhibited an approximately spherical shape, with an average diameter of around 13 µm, comparable to that of native starch microparticles. Importantly, the particles appeared more homogeneous and less prone to aggregation compared to systems obtained using poly (isobutylene-alt-maleic anhydride) alone. This observation suggests that pre-functionalization with tryptophan promoted a more controlled particle formation process, likely due to improved interfacial balance and steric stabilization imparted by the amino acid residues (**Figure 1.1.12**).

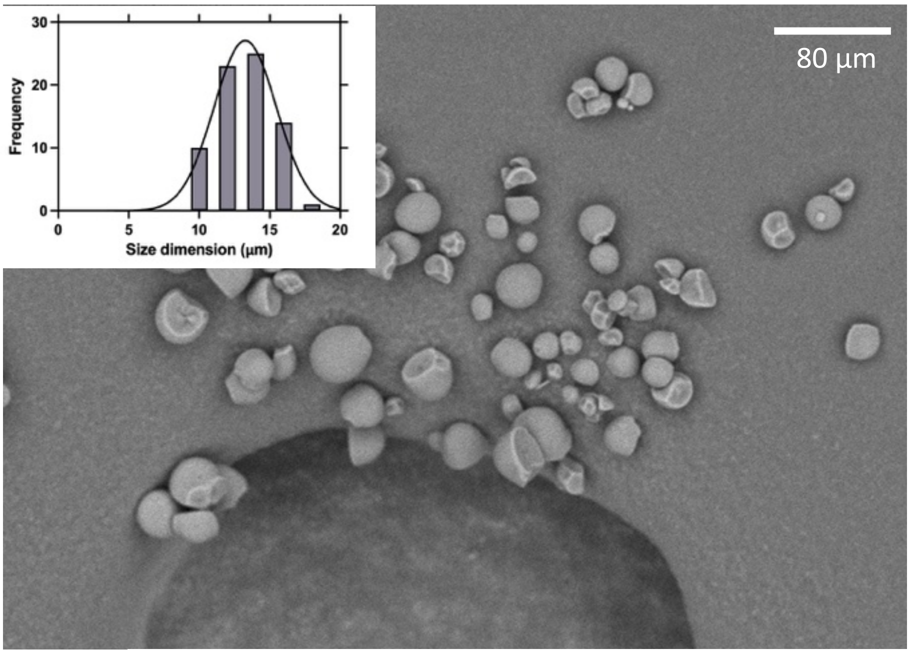

Figure 2.1.12 SEM image of starch PMW microparticles.

Overall, this sequential functionalization strategy, based on the use of Poly (isobutylene-alt-maleic anhydride) as a polymeric linker for amino acid grafting, proved to be an effective approach for tailoring the surface chemistry of starch microparticles. By combining the hydrophobic backbone of Poly (isobutylene-alt-maleic anhydride) with the structural and optical functionalities of tryptophan, the resulting microparticles display enhanced hydrophobicity. These features make them particularly attractive for cosmetic formulations, where improved skin adhesion, better compatibility with lipid phases, and multifunctionality are highly desirable.

In order to further explore the multifunctional potential of PMW-functionalized starch microparticles, a preliminary assessment of their UV-protective performance was carried out in collaboration with Abich Srl (Milan, Italy). This evaluation was intended as a screening tool to support formulators during the early stages of product development, before performing *in vivo* sun protection factor (SPF) determination in accordance with ISO 24444:2019. The PMW-functionalized starch microparticles exhibited a measured SPF value of 2.2, indicating a low but detectable UV-protective effect. Although this value is insufficient to classify the material as an effective standalone sunscreen agent, it confirms the contribution of tryptophan residues to UV absorption, particularly in the UVB region. These findings suggest that the modified starch powder could potentially act as a secondary or auxiliary UV-absorbing component in cosmetic formulations, rather than as a primary UV filter. Importantly, this preliminary result supports the conceptual strategy of introducing UV-active moieties into biopolymer-based microparticles to impart additional functional benefits beyond textural modification.

Given the promising physical appearance and tactile properties of the PMW-modified starch powder, a sensory evaluation was also conducted to assess its cosmetic performance from a user perspective. A panel of 27 volunteers participated in a comparative sensory test aimed at evaluating key attributes such as spreadability, softness, and absorption. The results indicated that the chemical modification exerted a selective influence on the sensory profile of modified tapioca starch. No statistically significant differences were observed in spreadability or softness (ns), suggesting that the modification did not adversely affect the mechanical behavior of the powder during application or the tactile sensation after deposition on the skin. In contrast, a statistically significant increase in absorption was observed for the PMW-modified starch compared to the native material ($p < 0.0001$). (**Figure 2.1.13**).

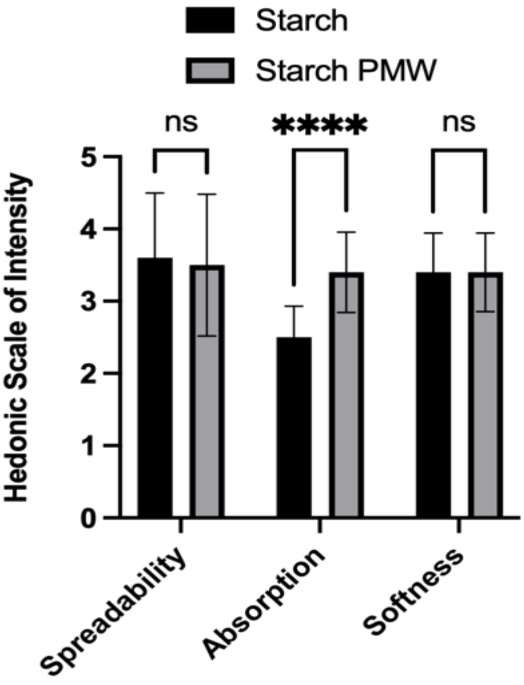

Figure 2.1.13 Sensory evaluation of spreadability, absorption, and softness of Starch and Starch PMW. Data are expressed as mean ± SD. Statistical significance is indicated as ns or **** ($p < 0.0001$).

This finding indicates an enhanced interaction between the modified particles and skin moisture, which may be attributed to the combined presence of hydrophobic domains and polar functional groups introduced through the PMW functionalization. Notably, this increase in absorption occurred without compromising handling or sensory comfort, highlighting the potential of this modification strategy to improve functional performance while preserving favorable sensorial attributes.

Overall, the combined SPF screening and sensory evaluation demonstrate that PMW-functionalized starch microparticles exhibit a balanced profile of functional and sensorial properties. While their intrinsic UV protection remains limited, the enhanced absorption behavior and preserved tactile qualities support their potential use as multifunctional, bio-based ingredients in cosmetic formulations, particularly as texture modifiers or supplementary functional fillers in skin-care and sun-care products.

2.2. Preparation of Chitosan MPs: Synthesis and characterization

Following the investigation on starch-based microparticles, the study was extended to other widely used natural polymers in cosmetic formulations, chitosan and carboxymethyl cellulose (CMC), in order to evaluate the versatility of the functionalization strategies and particle production methods across different polysaccharide matrices.

Chitosan was selected due to its well-known biocompatibility, biodegradability, film-forming ability, and intrinsic affinity for skin and hair substrates. However, its highly hydrophilic nature and limited compatibility with lipid-rich cosmetic formulations often restrict its use as a multifunctional ingredient [61]. For this reason, a hydrophobic modification strategy based on oleic acid was also applied to chitosan, with the aim of improving its interfacial properties, skin adhesion, and compatibility with oily phases.

Unlike starch, chitosan was preferentially processed into microparticles using spray drying. This choice was driven by the rheological behavior of chitosan solutions: when dissolved and activated in dilute acetic acid, chitosan forms a viscous, homogeneous solution that is particularly suitable for atomization. The spray-drying process enables rapid solvent evaporation and direct conversion of the polymer solution into dry microparticles, minimizing aggregation phenomena and ensuring good control over particle morphology.

Before spray drying, chitosan was activated in acetic acid and reacted with oleic acid under continuous stirring for 24 h. Oleic acid was introduced to enhance the hydrophobic character of the chitosan matrix through ionic and hydrogen bonding interactions between its carboxylic group and the amino functionalities of chitosan (**Figure 2.2.1**). This modification intended to improve skin adhesion, moisture retention, and overall compatibility with lipid-based cosmetic formulations. The resulting solution was subsequently fed into the spray dryer, where solvent evaporation led to the formation of oleic-acid-modified chitosan microparticles.

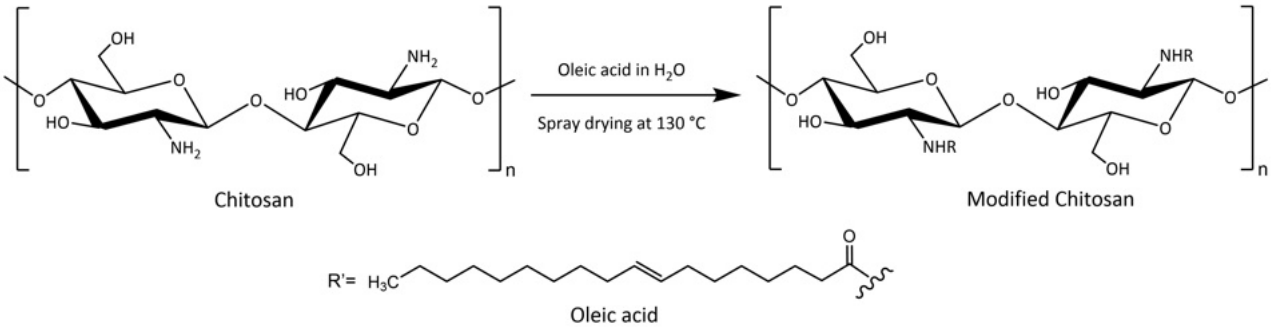

Figure 2.2.1 Schematic representation of the chemical reaction between chitosan and oleic acid.

FT-IR analysis of the spray-dried chitosan microparticles provided clear evidence of successful functionalization. The spectrum of the modified sample displayed a characteristic absorption band at approximately 1730 cm^{-1}, corresponding to the C=O stretching vibration of the carboxylic group associated with oleic acid. In addition, a marked increase in the intensity of the aliphatic C–H stretching bands in the 2920–2850 cm^{-1} region was observed, reflecting the incorporation of the long hydrocarbon chain of oleic acid. A concomitant decrease in the intensity of the N–H bending vibration around 1590 cm^{-1} was also detected, consistent with the involvement of chitosan amino groups in interactions or partial amide bond formation with the carboxyl functionality of oleic acid. Overall, these spectral features strongly suggest that oleic acid is not merely physically adsorbed but chemically associated with the chitosan backbone (**Figure 2.2.2**).

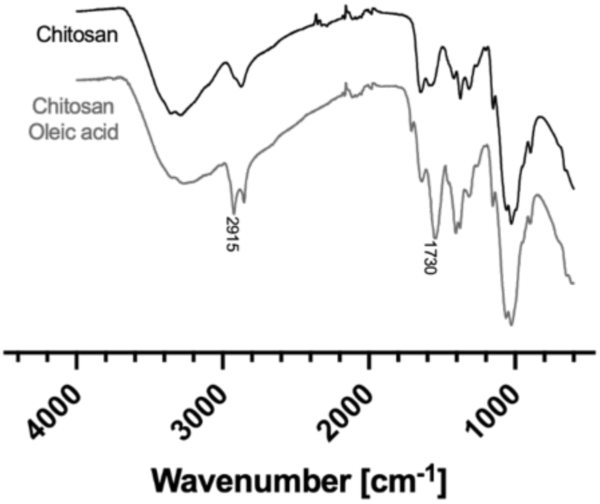

Figure 2.2.2 FT-IR spectra of modified chitosan with oleic acid.

The morphology of the resulting microparticles was examined by SEM. The spray-dried chitosan microparticles exhibited a predominantly spherical to slightly wrinkled morphology, characteristic of particles obtained via spray drying. The mean particle diameter ranged between 5 and 10 µm, falling within the size range relevant for cosmetic applications. The particles appeared relatively uniform, with a low degree of aggregation, indicating that the atomization and drying parameters were appropriately optimized. Moreover, the surface morphology suggested the formation of compact, dense particles with limited porosity, in agreement with the increased hydrophobicity imparted by oleic acid functionalization (**Figure 2.2.3).**

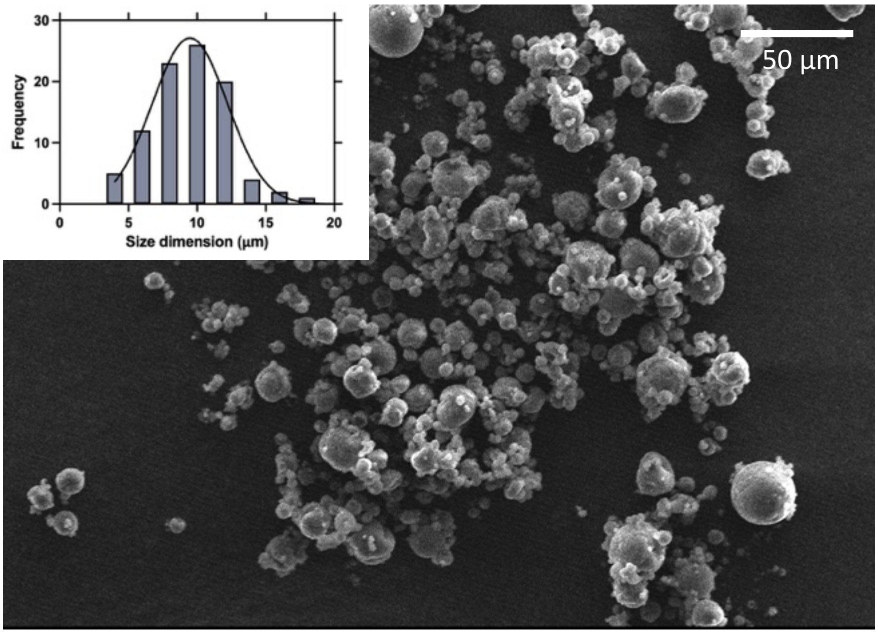

Figure 2.2.3. SEM photograph of Chitosan oleic acid microparticles.

For chitosan, the chemical modification with oleic acid resulted in a marked and consistent improvement across all evaluated attributes. As shown in **Figure 2.2.4**, modified chitosan received significantly higher scores for spreadability, absorption, and softness compared with the native material ($p < 0.0001$ for all comparisons). This uniform enhancement suggests that functionalization substantially alters particle skin interactions, improving both tactile behavior during application and the perceived finish on the skin.

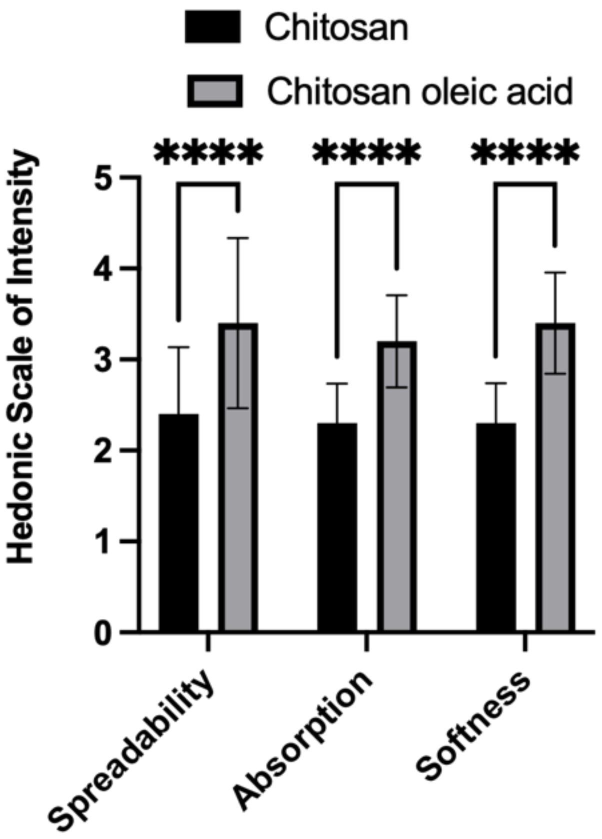

Figure 2.2.4. Sensory evaluation of spreadability, absorption, and softness of Chitosan and Chitosan oleic acid. Data are expressed as mean ± SD. Statistical significance is indicated as ns or **** ($p < 0.0001$).

2.3. Preparation of CMC MPs: Synthesis and characterization

Similarly to chitosan, sodium carboxymethyl cellulose (Na-CMC) was functionalized with oleic acid (**Figure 2.3.1**) and processed under optimized spray-drying conditions to investigate the applicability of this strategy to cellulose-based polymers. Na-CMC was selected due to its widespread use in cosmetic formulations as a thickener, stabilizer, and film-forming agent, as well as for its excellent biocompatibility and high density of carboxyl and hydroxyl functional groups. However, its strongly hydrophilic character often limits its performance in formulations containing lipidic components. Therefore, the introduction of oleic acid was pursued as a means to modulate the hydrophilic–hydrophobic balance of Na-CMC and enhance its interfacial and film-forming properties in cosmetic applications [31].

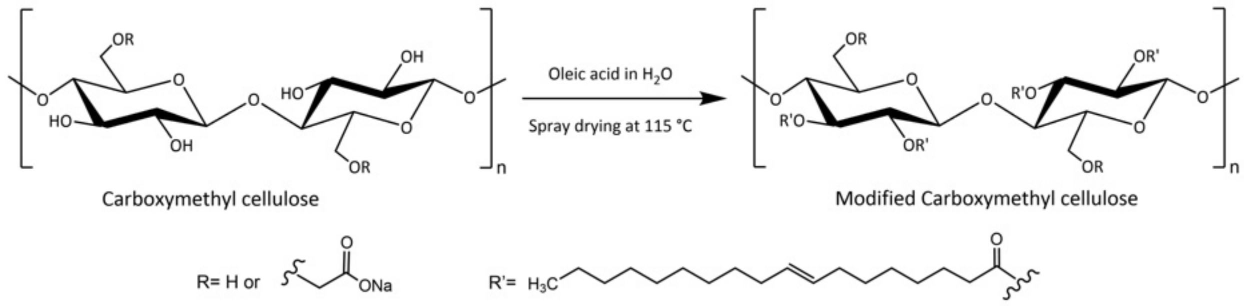

Figure 2.3.1. Schematic representation of the chemical reaction between Carboxymethylcellulose and oleic acid.

FT-IR spectroscopy provided clear evidence of the successful association of oleic acid with the Na-CMC matrix. The spectra of the functionalized microparticles exhibited characteristic absorption bands at approximately 2915 cm^{-1}, attributable to the CH_2 stretching vibrations of the aliphatic chains of oleic acid, and at around 1730 cm^{-1}, corresponding to the C=O stretching of the carboxylic group. The presence and partial overlap of these bands with those of the cellulose derivative indicate that oleic acid was effectively incorporated into the polymer network, most likely through a combination of ionic interactions between the carboxylate groups of Na-CMC and the protonated carboxylic acid of oleic acid, as well as hydrogen bonding with hydroxyl groups along the cellulose backbone (**Figure 2.3.2**). These interactions are consistent with a stable modification rather than simple physical adsorption.

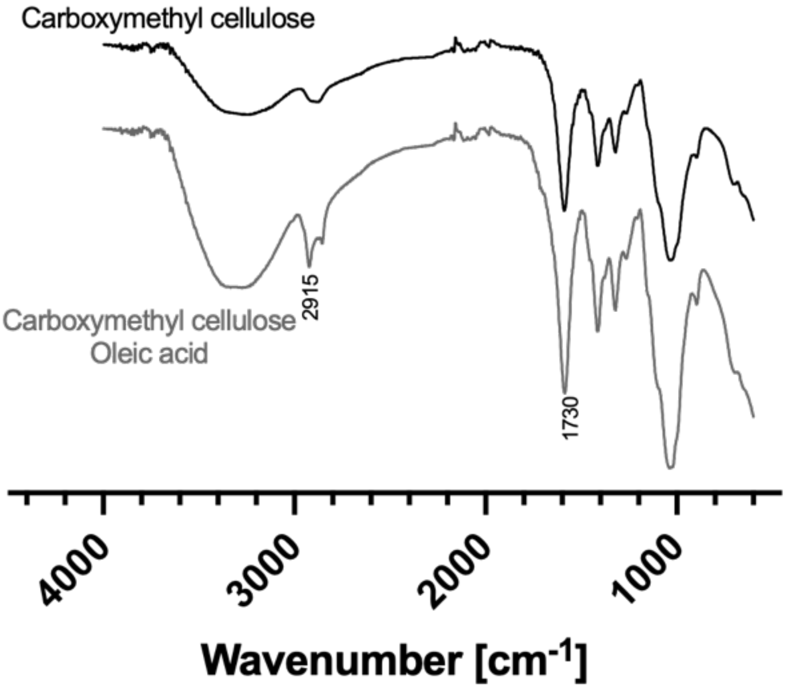

Figure 2.3.2 FT-IR spectra of Carboxymethyl cellulose oleic acid.

SEM analysis revealed that the spray-dried Na-CMC microparticles exhibited a smooth and predominantly spherical morphology, with an average particle diameter of approximately 5 µm. The narrow size distribution and regular surface texture suggest efficient atomization and rapid solvent evaporation during the spray-drying process (**Figure 2.3.3**).

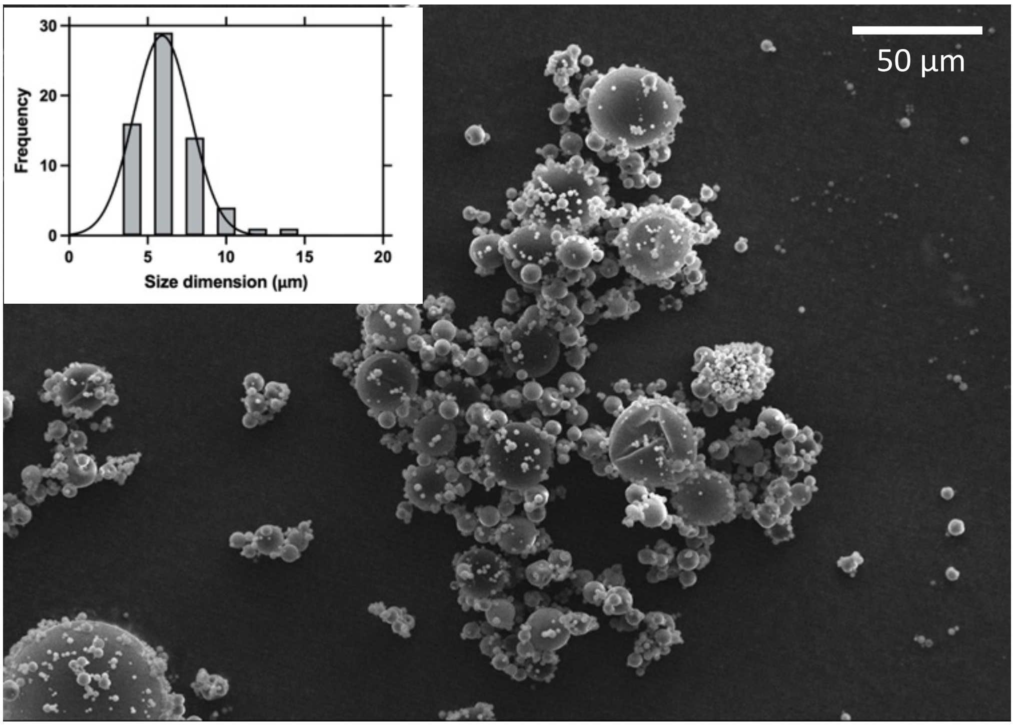

Figure 2.3.3 SEM image of carboxymethyl cellulose oleic acid microparticles.

From a cosmetic perspective, these morphological features are particularly advantageous, as smooth and spherical particles are known to improve powder flowability, dispersibility, and sensory perception upon topical application. Moreover, the relatively small and uniform particle size is expected to facilitate homogeneous incorporation into emulsions and gels, contributing to improved aesthetic performance and formulation stability.

Overall, spray drying proved to be a robust, solvent-efficient, and scalable approach for the production of bio-based microparticles from cellulose derivatives. The precise control of key processing parameters, such as feed rate, inlet temperature, and drying gas flow, enabled fine-tuning of particle size and morphology across different polymer systems. In addition, the incorporation of oleic acid emerged as a versatile and effective strategy to modify the surface properties of hydrophilic polysaccharides, enhancing their compatibility with lipid-containing formulations and broadening their potential application as functional alternatives to synthetic microplastics in cosmetic products.

From a sensorial point of view, CMC showed a response pattern similar to modified chitosan, with significant improvements observed in all three sensory attributes ($p < 0.0001$ for spreadability, absorption, and softness). The modified CMC scored consistently higher than the native powder, reflecting improved ease of distribution on the skin, faster perception of absorption, and a softer tactile finish (**Figure 2.3.4**).

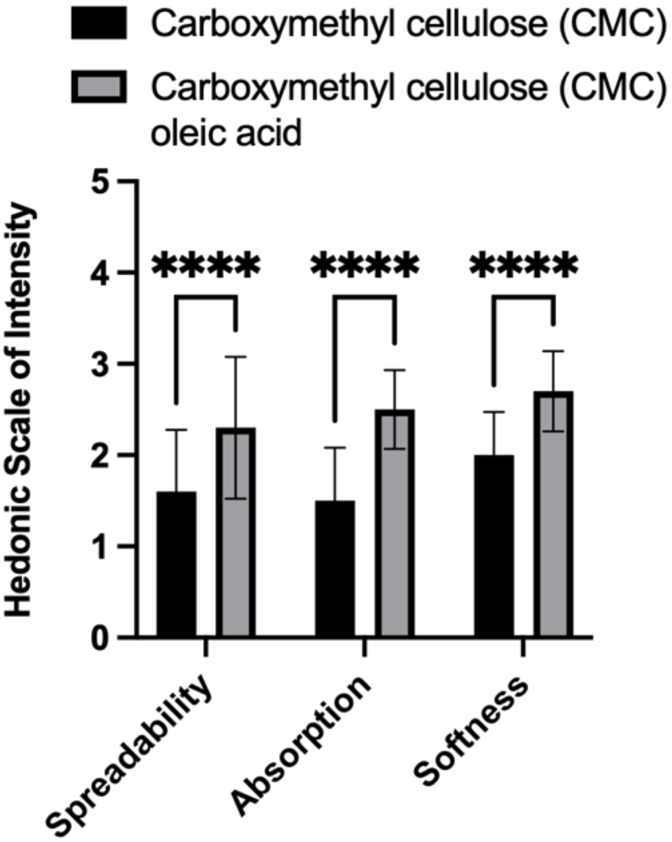

Figure 2.3.4 Sensory evaluation of spreadability, absorption, and softness of CMC and CMC oleic acid. Data are expressed as mean ± SD. Statistical significance is indicated as ns or **** ($p < 0.0001$).

Collectively, these findings indicate that chemical modification can substantially alter the sensory performance of biopolymer-based powders, with the magnitude and nature of the improvement depending on the chemical structure of the base polymer.

3. Discussion

The present work aimed to develop sustainable, bio-based polymeric microparticles with tailored surface properties by exploiting natural polysaccharides and mild chemical functionalization strategies. In particular, starch, chitosan, and sodium carboxymethyl cellulose were selected as model biopolymers due to their abundance, biodegradability, and established use in cosmetic formulations.

A first aspect concerns the method used for microparticle production. Precipitation and spray drying were systematically evaluated, revealing distinct advantages and limitations depending on the polymer system. For starch-based systems, precipitation emerged as the most suitable technique. The controlled addition of ethanol as an antisolvent enabled the formation of well-defined microparticles within a close concentration range (20–40% v/v), where solvent–nonsolvent equilibrium favored nucleation over aggregation. This behavior can be explained by the fundamental mechanisms of precipitation, which involve rapid mixing of a polymer solution with a non-solvent, leading to supersaturation, nucleation, and subsequent particle growth. According to recent literature, the degree of supersaturation plays a critical role in determining particle size and uniformity, with higher supersaturation promoting the formation of smaller and more homogeneous particles [63]. In this context, the identified ethanol concentration window likely represents an optimal balance between solvent diffusion and polymer desolvation, enabling controlled nucleation while limiting uncontrolled aggregation.

Furthermore, precipitation is widely recognized as a kinetically controlled process in which particle size and morphology are governed by the interplay between mixing dynamics and solvent properties [64]. During the early stages of mixing, polymer chains form dynamic aggregates that grow until reaching a critical solvent composition, beyond which further growth is arrested. This mechanism is consistent with the formation of stable and well-defined microparticles observed in this study, suggesting that the selected precipitation conditions effectively control both nucleation and growth processes. In contrast, spray drying of starch solutions, particularly those functionalized with oleic acid, led to hollow or collapsed particles and relatively low powder recovery, likely due to rapid solvent evaporation and poor film-forming capability of gelatinized starch. This behavior can be explained by the fundamental mechanism of spray drying, which involves atomization of a liquid feed into fine droplets followed by rapid solvent evaporation. During this process, the formation of a solid shell at the droplet surface, coupled with internal solvent diffusion, can induce structural stress, leading to particle shrinkage, deformation, or collapse. Such phenomena are well documented for polysaccharide-based systems, where limited film-forming ability and low mechanical strength of the polymer matrix can result in hollow or irregular morphologies. Chemical functionalization proved to be a key strategy to overcome the intrinsic hydrophilicity of native starch. The introduction of oleic acid significantly altered both the surface chemistry and the handling properties of the resulting microparticles. These results highlight that, unlike precipitation, spray drying is strongly dependent on the physicochemical properties of the polymer solution, including viscosity, concentration, and drying behavior. While spray drying is widely recognized as a scalable and industrially relevant technique, its application to starch-based systems requires careful optimization of formulation parameters to ensure particle integrity and process efficiency.

FT-IR analysis confirmed the presence of ester or carboxyl-related bands, while SEM observations indicated the preservation of particle integrity following modification. These results suggest that even low degrees of hydrophobic grafting are sufficient to modify interfacial behavior without compromising particle morphology. The use of poly (isobutylene-alt-maleic anhydride) as a polymeric linker further expanded the functional versatility of starch microparticles. Poly (isobutylene-alt-maleic anhydride) was selected not only for its availability and biocompatibility, but also for the high reactivity of its repeating succinic anhydride units. Functionalization using poly (isobutylene-alt-maleic anhydride) introduced additional hydrophobic domains while providing reactive sites for covalent interactions with starch hydroxyl groups. Spectroscopic changes in the carbonyl and carbohydrate fingerprint regions confirmed successful modification, while SEM analysis revealed more uniform and less aggregated particles compared to poly (isobutylene-alt-maleic anhydride). This suggests that pre-functionalization with an amino acid can positively influence particle formation and stability.

Among the amino acids investigated, tryptophan represents a particularly interesting functional moiety. Its aromatic indole ring contributes to increased hydrophobicity. Sensory evaluation revealed that the chemical modification of starch selectively influenced absorption behavior without negatively affecting spreadability or softness. The increased absorption observed for PMW-modified starch suggests enhanced interaction with skin moisture, an attribute that is particularly desirable in cosmetic powders. This highlights a crucial point: functionalization strategies can be designed to improve specific performance parameters while preserving favorable tactile properties.

For both chitosan and Na-CMC systems, spray drying proved highly effective, yielding spherical, uniform microparticles with controlled size distributions. Oleic acid functionalization of chitosan resulted in chemically grafted structures, as evidenced by FT-IR, and dense particle morphologies consistent with increased hydrophobicity. Similarly, Na-CMC microparticles displayed smooth surfaces and small size distributions, features that are advantageous for topical application and aesthetic performance.

Overall, this study demonstrates that simple, scalable, and solvent-compatible strategies can be employed to modulate the surface properties of natural biopolymer microparticles. While the functional enhancements achieved here are modest, they provide a strong foundation for future optimization. Based on the physicochemical and functional properties discussed in this chapter, Chapter 2 will address the formulation study, exploring the incorporation of the modified biopolymer microparticles into cosmetic emulsions and evaluating their influence on formulation stability, texture, and skin-related performance.

4. Materials and Methods

4.1. Reagents

Tapioca starch was purchased from Nouryon Chemicals SPA (Milan, Italy). Poly (isobutylene-alt-maleic anhydride) MW 6 KDa, Tryptophan, Chitosan low molecular weight, Carboxymethylcellulose sodium salt, Oleic acid were purchased from Sigma-Aldrich (St. Louis, MI, USA). N,N-Dimethylformammide (DMF), Acetic acid, Ethanol, and 1-Octanol were purchased from CARLO ERBA Reagents s.r.l. (Milan, Italy).

4.2. Synthesis of sustainable microparticles

Synthesis of Tapioca Starch microparticles with oleic acid

Tapioca starch (5 g, 100 g L^{-1}) was dispersed in 50 mL of distilled water and heated to 50 °C. After 30 min, oleic acid (0.285 mL, 0.05% w/v) was added, and the mixture was stirred at 50 °C for 4 h to promote starch-lipid complex formation. Microparticles were obtained by precipitating the aqueous phase dropwise into 50 mL of a water/ethanol solution (20% v/v) under continuous stirring for 30 min. The resulting suspension was filtered through a Büchner funnel equipped with a 200 nm cellulose filter. The collected microparticles were washed by centrifugation at 1000 × *g* for 20 min to remove excess oleic acid, followed by lyophilization.

Synthesis of Tapioca Starch microparticles functionalized with Poly (isobutylene-alt-maleic-anhydride)

Tapioca starch (5 g, 100 g L^{-1}) was dispersed in 50 mL of distilled water and heated to 50 °C. After 30 min, Poly (isobutylene-alt-maleic-anhydride) (0.05% w/v was added, and the mixture was stirred at 50 °C for 2 h. Microparticles were obtained by precipitating the aqueous phase dropwise into 50 mL of a water/ethanol solution (20% v/v) under continuous stirring for 30 min. The resulting suspension was filtered through a Büchner funnel equipped with a 200 nm cellulose filter. The collected microparticles were washed by centrifugation at 1000 × *g* for 20 min to remove excess Poly (isobutylene-alt-maleic-anhydride) followed by lyophilization.

Synthesis of Tapioca Starch microparticles functionalized with Poly (isobutylene-alt-maleic-anhydride) by spray drying

Tapioca starch (5 g, 100 g L^{-1}) was dispersed in 50 mL of distilled water and heated to 90 °C. After 30 min, Poly (isobutylene-alt-maleic-anhydride) (0.05% w/v) was added, and the mixture was stirred at 90 °C for 1h. After, the solution was spray-dried under the following conditions: feed rate 3 mL min^{-}

^{1} (10%), inlet temperature 130 °C, outlet temperature 90 °C, air pressure 6.0 bar, aspiration rate 100%, nozzle clear 2.

Synthesis of Tapioca Starch microparticles with oleic acid by spray drying

Tapioca starch (5 g, 100 g L^{-1}) was dispersed in 50 mL of distilled water and heated to 90 °C. After 30 min, oleic acid (0.285 mL, 0.5% w/v) was added, and the mixture was stirred at 90 °C for 90 min to promote starch-lipid complex formation. After, the solution was spray-dried under the following conditions: feed rate 3 mL min^{-1} (10%), inlet temperature 170 °C, outlet temperature 102 °C, air pressure 6.0 bar, aspiration rate 100%, nozzle clear 2.

Synthesis of Tapioca Starch microparticles in 1-octanol

The procedure was similar to the previous method, except that the aqueous starch solution was precipitated into 30 mL of octanol (100% v/v) and stirred for 30 min. Microparticles were recovered by centrifugation at 1000 × *g* for 20 min and lyophilized. Octanol, being immiscible with water, was recovered and reused.

Synthesis of Tapioca Starch microparticles functionalized with Tryptophan-grafted- Poly (isobutylene-alt-maleic anhydride) (PMW)

PMW polymer was synthesized by dissolving Poly (isobutylene-alt-maleic anhydride) (1 g) and Tryptophan (464 mg) in DMF (15 mL) at 60 °C overnight. The polymer was purified with 0.3 M HCl, centrifuged at 3200 × *g* for 10 min, and lyophilized after freezing. Tapioca starch (5 g) and PMW (250 mg) were dispersed in 50 mL of distilled water at 50 °C for 2 h. Microparticles were formed by dropwise precipitation into 50 mL of ethanol (20% v/v), stirred for 30 min, filtered through a 200 nm cellulose filter, frozen and lyophilized.

Synthesis of Chitosan microparticles

Chitosan (10 g L^{-1}) was dissolved in 50 mL of 1% (v/v) acetic acid at room temperature under stirring for 24 h. Oleic acid (0.285 mL, 0.5% w/v) was added, and the solution was spray-dried under the following conditions: feed rate 3 mL min^{-1} (10%), inlet temperature 130 °C, outlet temperature 90 °C, air pressure 6.0 bar, aspiration rate 90%, nozzle clear 2.

Synthesis of Carboxymethyl Cellulose Sodium Salt (Na-CMC) Microparticles

Na-CMC (10 g L^{-1}) was dissolved in 60 mL of ultrapure water under stirring at room temperature for 24 h. Oleic acid (0.342 mL, 0.5% w/v) was added, and the mixture was stirred at 400 rpm for 2 h.

The solution was spray-dried under the following conditions: feed rate 4 mL min^{-1} (15%), inlet temperature 115 °C, outlet temperature 64 °C, air pressure 6.0 bar, aspiration rate 90%, nozzle clear

4.3. Microparticles characterization

Fourier Transform Infrared (FT-IR) Spectroscopy

Chemical structure and functional groups of native and modified powder were analyzed using FT-IR (FT-IR spectrometer platform INVENIO, Bruker Optics GmbH & Co. KG) in transmission mode over 500–4000 cm^{-1}, averaging 16 scans at 2 cm^{-1} resolution. Samples subjected to analysis were positioned on a diamond crystal sampling plate and fixed in place with a pointed clamp. A background spectrum was collected before each sample scan using an empty plate. The ATR crystal and pointed tip were cleaned between measurements to eliminate any interference from previous samples.

Scanning Electron Microscopy (SEM)

For scanning electron microscopy (SEM) analysis, the microparticle samples were mounted on conductive double-sided carbon adhesive tape attached to aluminum stubs. The samples were sputter-coated with a thin layer of gold and examined using a scanning electron microscope (Phenom Pro G6, Thermo Fisher Scientific) operated at an accelerating voltage of 10.0 kV. Photomicrographs were acquired at various magnifications to assess homogeneity, and particle diameters were measured using the ImageJ particle analyzer plugin.

Consumer evaluation of the sensory appeal of powders

A preliminary consumer sensory evaluation was conducted on 3 powders (starch, chitosan, and carboxymethylcellulose, both in their native and chemically modified forms). The test was carried out on a panel of 27 volunteers of both sexes. Each participant received a questionnaire in which five parameters had to be evaluated: color, consistency, spreadability, absorption, and softness. Specifically, color and consistency were assessed visually before powder application on the forearm, spreadability and absorption were evaluated during application, while softness was judged immediately after application. Each parameter was scored on a structured scale. For color and consistency, participants were asked to give a categorical judgment. For spreadability, absorption, and softness, a 4-point scale was used, where 1 represented the lowest intensity (absence of the characteristic) and 4 the highest intensity. The powders were presented to participants in designed plastic trays, each coded with a two-letter random code unknown to the panelists, in order to minimize external influences. Before starting, participants were instructed to clean their hands and forearms with paper. They were then shown how to apply the powders on the forearm using circular movements until the sample was fully spread. Immediately afterward, participants filled in the questionnaire according to the predefined evaluation phases.

The results and the data collected were elaborated with two-way ANOVA analysis at a significance level of $p < 0.05$ to determine the statistical differences between the powder samples, based on the characteristics described by the panelists.

In vitro preliminary evaluation of the sunscreen protection factor

The test for the preliminary evaluation of the sun protection factor (SPF) is a method developed and validated by Abich Srl. The method aims to provide a tool to support formulators in obtaining a preliminary assessment of the product, before performing in vivo tests according to ISO 24444:2019. Sample preparation and testing were conducted following the requirements for in vitro sunscreen evaluation outlined in ISO 24443:2021.

A fixed amount of the PMW-modified starch powder was applied onto polymethyl methacrylate (PMMA) plates (Schönberg R6, batch REW5-221207mw-1382), which comply with the optical transmittance and surface roughness specifications defined by the standard. The powder was evenly distributed on the substrate to obtain a homogeneous layer. After drying, the samples were analyzed using a Labsphere® UV-2000S spectrophotometer, allowing the determination of the sun protection factor over the 290–400 nm wavelength range, encompassing both UVB and UVA regions. SPF values were calculated by the dedicated Labsphere® UV-2000S software using the correction factors and equations specified in ISO 24443.

5. Bibliography

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Chapter 2: Emulsion systems for cosmetic applications: fundamentals, stability, and rheological control

The formulation study presented in this chapter was carried out during a research period abroad at AgroParisTech Chaire de Cosmétologie (Orléans, France), under the supervision of Prof. Delphine Huc-Mathis and Prof. Richard Daniellou.

1. Background

1.1. Emulsion Systems in Cosmetic Formulations

Due to consumer demand for safe and environmentally-friendly products with a 'clean-label', sustainable cosmetics gaining popularity in both industrial and academic sectors [1].

A cosmetic product is generally defined as a substance or mixture intended for external application to the human body with the primary aim of cleansing, perfuming, modifying appearance, protecting, or maintaining the body in good condition, without exerting therapeutic or disease-preventive effects. In this context, skincare products are a specific category of cosmetics designed for application to the epidermis. The efficiency of their preparation is influenced by both the ingredients and the technologies employed. Based on their physical state, skincare formulations can be classified into three main groups: liquid systems (such as solutions or suspensions), solid forms (including powders), and semisolid systems (gels and emulsions). Emulsions are the most frequently used and can be classified as creams or lotions depending on their consistency [2], [3]. Emulsions play an essential role in the beauty and personal care industry, particularly in skin and hair care applications, due to their remarkable versatility and tunable properties, which make them the most commonly used form of commercial products [4]. From a colloidal science perspective, emulsions are defined as thermodynamically unstable dispersions formed by two immiscible liquids, where one of them is dispersed as droplets (dispersed phase) into the second one (continuous phase) [5]. Depending on the nature of the two phases, their mutual interactions, and the size of the dispersed droplets, a wide variety of emulsion systems can be formulated [6].

The high surface energy of the interface between the two immiscible phases causes thermodynamic instability, which leads to emulsion destabilization through different processes, such as flocculation, creaming, coagulation, coalescence, phase inversion, and Ostwald ripening [7]. As a consequence, emulsions require appropriate stabilization strategies to ensure adequate shelf life and resistance to environmental stresses. This is commonly achieved through the addition of emulsifiers, often used as blendsor by exploiting electrostatic and steric stabilization mechanisms.

The classification of emulsions is generally based on specific parameters, including the arrangement and number of aqueous and oil phases, as well as the size and concentration of droplets and the type of stabilizer used to reduce the interfacial tension between the liquids [8].

Based on the arrangement of the aqueous and oil phases, simple emulsions can be classified as oil-in-water (O/W) or water-in-oil (W/O) systems [9].

Beyond these conventional structures, more complex architectures can be obtained by combining simple emulsions, leading to so-called multiple emulsions. Among these, water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O) systems are of particular interest. In a W/O/W emulsion, water droplets are initially dispersed within an oil phase to form a primary W/O emulsion, which is subsequently re-dispersed into an external aqueous phase, resulting in a hierarchical droplet organization.

From a formulation point of view, emulsion stability and performance are reached by adding stabilizers, such as emulsifiers (surfactants, proteins) or solid particles. These compounds can improve the systems stability over time by retarding or preventing destabilization [10], [11]. Conventional cosmetic emulsions commonly rely on surfactants such as sodium lauryl sulfate, sodium dodecyl sulfate, sodium laureth sulfate, polyethylene glycol ethers, polysorbate 80, and sorbitan oleate (Span 80) [12]. Despite their widespread use, growing evidence has highlighted potential drawbacks associated with these compounds, including adverse effects on both human health and the environment. Several studies have reported surfactant-induced cytotoxicity, hemolytic activity, and skin irritation [13]. In particular, surfactants employed as emulsifiers or cleansing agents have been shown to trigger undesirable cutaneous responses, such as inflammation and contact dermatitis [14].

In this context, Pickering emulsions, stabilized by solid particles irreversibly adsorbed at the oil–water interface, have emerged as a promising alternative to conventional surfactant-based systems (**Figure 1.1.1**). Owing to their ability to stabilize emulsions without the need for molecular emulsifiers, Pickering emulsions are often regarded as more sustainable and environmentally friendly formulations [15]. Consequently, they have attracted increasing interest in both food and cosmetic applications. Pickering emulsions stabilized by biopolymer-based particles have gained significant attention for cosmetic and skincare uses due to their non-toxic nature, biocompatibility, and biodegradability, making them well aligned with current demands for safer and more sustainable cosmetic formulations [16].

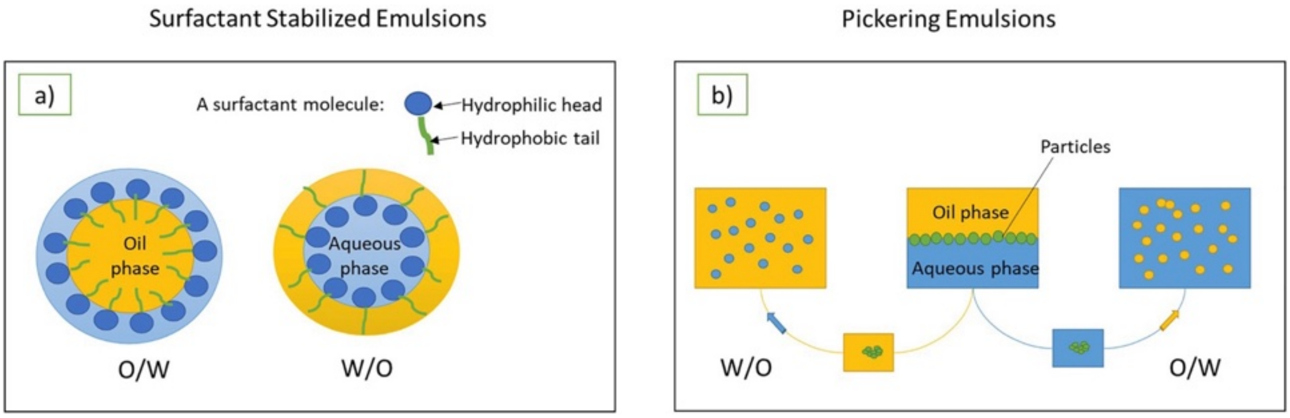


Figure 1.1.1 Schematic representation of the key features distinguishing surfactant-stabilized emulsions (a) from particle-stabilized (Pickering) emulsions (b) [12] .

1.2. Pickering Emulsion

A Pickering emulsion is a distinctive emulsion described by Ramsdem and Pickering more than one century ago and have undergone significant development in the last two decades [17], [18].

In contrast to conventional emulsions, Pickering emulsions are a type of emulsion stabilized by fine solid particles rather than by surfactants. Solid particles possessing appropriate wettability can adsorb at the oil–water interface, where they spontaneously accumulate due to their dual affinity for both phases. Upon adsorption, the particles reorganize on the droplet surface to form a dense interfacial layer, which may consist of a single or multiple particle layers. This interfacial film stabilizes the emulsion droplets by providing steric hindrance and volume exclusion, thereby effectively preventing droplet coalescence. In Pickering emulsions, the nature of the continuous phase is determined by the liquid that preferentially wets the particle surface, while the less-wetting liquid constitutes the dispersed phase [16]. The use of solid particles as stabilizing agents enables fine control over emulsion characteristics, including emulsion type, droplet organization, and viscosity, while also influencing sensory attributes such as texture, appearance, and skin feel upon application. This versatility facilitates the development of innovative cosmetic formulations and multifunctional delivery systems. Furthermore, compared to conventional surfactant-stabilized emulsions, Pickering emulsions exhibit several advantageous properties, including enhanced resistance to droplet coalescence, reduced susceptibility to Ostwald ripening, improved biocompatibility, and low cytotoxicity.

In fact, the use of particles allows controlling the nature of the emulsions, the organization of the droplets and viscosity, modifying the sensory feeling upon application, product appearance and texture [19]. This enables the development of innovative cosmetic formulations and multifunctional delivery systems. Moreover, Pickering emulsions offer many interesting properties compared to conventional ones, including superior stability against coalescence, minimization of the Ostwald ripening phenomena, higher biocompatibility and low cytotoxicity [20].

A wide variety of solid particles has been employed as stabilizers for Pickering emulsions, including inorganic materials such as silica and calcium carbonate, carbon-based materials such as graphene, polysaccharides (e.g., cellulose, chitosan, and starch), plant-derived proteins (from lentil, chickpea, lupin, and soy), polyphenol crystals, as well as synthetic polymer particles [21], [22].

The effectiveness of solid particles as Pickering stabilizers is largely governed by their wettability and structural characteristics. Ideally, particles should exhibit partial affinity for both oil and water phases to ensure stable interfacial adsorption. However, many naturally occurring solid particles are intrinsically hydrophilic, which limits their emulsifying efficiency. Consequently, surface modification strategies, such as esterification or grafting with hydrophobic moieties, are often employed to tailor particle wettability and enhance their performance in Pickering emulsions. In general, amphiphilic characteristics are advantageous, as they promote particle adhesion at the oil–water interface and enable modulation of interfacial properties [23].

The wettability of solid particles, determined by the contact angle in water (θw) at the oil-particle-water interface, indicates the type of Pickering emulsion formed. Particle with a contact angle below 90° are hydrophilic and, according to the Bancroft rule, tend to stabilize O/W emulsions. In contrast, if the contact angle is above 90°, the particle is hydrophobic and allow to stabilize W/O emulsions [24], [25] (**Figure 1.2.1**). Optimal stabilization is typically achieved when the contact angle approaches 90°, as excessively hydrophilic or hydrophobic particles tend to remain dispersed in one phase rather than residing at the interface. Surface functionalization therefore, represents an effective strategy to fine-tune particle wettability and control emulsion type [23].

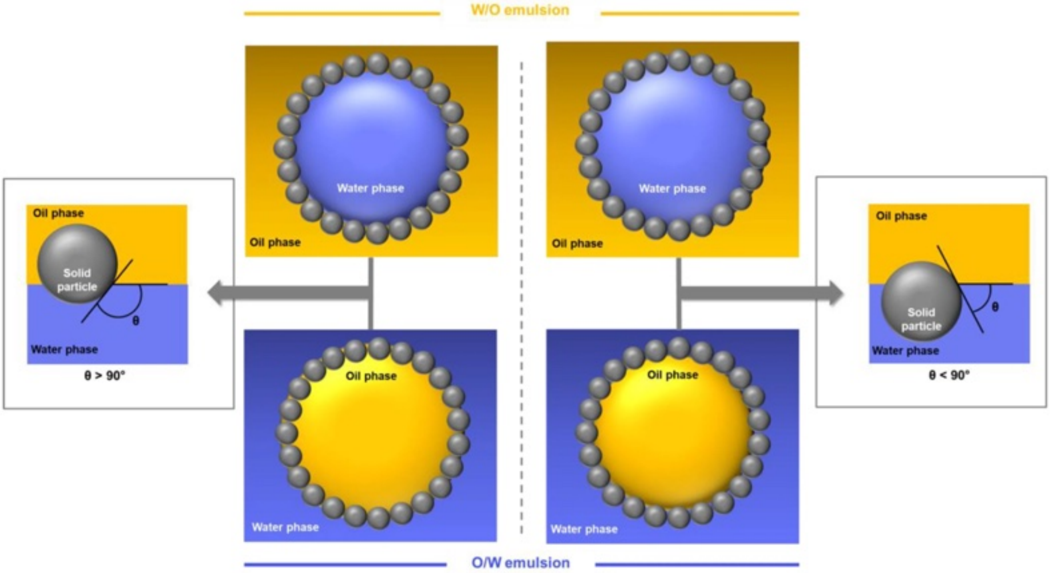


Figure 1.2.1 Schematic representations of spherical particles at the oil–water interface, showing the contact angle (θ) measured within the aqueous phase (left and right), along with the corresponding emulsion types as a function of the volume fraction of each phase [61].

1.3. Destabilization mechanisms of emulsions

The term "emulsion stability" usually refers to the ability of an emulsion to withstand changes in its physicochemical properties. This equilibrium is the ability of the emulsion to maintain the stability of its physical characteristics, such as size, shape, rheology, morphology, and others, for a long period of time [26]. It is commonly observed that the properties of an emulsion may remain unchanged for a certain period of time. Although this concept does not correspond to a true thermodynamic equilibrium, emulsion properties inevitably evolve over time; therefore, emulsion stability is determined by the rate at which these changes occur. According to the definition of the interfacial free energy ($\Delta G = \gamma_{ow} \Delta A$, with ΔA represents the change in the total interfacial area of the system), the increase in interfacial area upon emulsification is thermodynamically unfavorable and may promote system destabilization. Consequently, this energetic penalty must be compensated by a negative contribution arising from particle adsorption at the interface. Particle trapping at the oil–water interface is an irreversible process that induces kinetic arrest of the system; as a result, Pickering emulsions are characterized by kinetic stability [27], [28], [29], [30]. The destabilization of Pickering emulsions is typically associated with macroscopic phase separation and may occur through different mechanisms, either individually or in combination. Figure **1.3.1** schematically illustrates the main destabilization pathways leading to phase separation. Droplets with a density higher than that of the continuous phase tend to settle, forming a separated liquid layer at the bottom of the emulsion, whereas droplets with a lower density rise to the top, resulting in the formation of an upper liquid layer. These gravity-driven separation processes are known as sedimentation and creaming, respectively, and their occurrence strongly depends on the nature of the dispersed phase [31] (**Figure 1.3.1**). In addition, emulsion destabilization can occur through flocculation, in which droplets associate into larger aggregates without undergoing true coalescence. Ostwald ripening is particularly relevant in polydisperse emulsions and involves the growth of larger droplets at the expense of smaller ones via mass diffusion through the continuous phase, driven by differences in Kelvin pressure. Another important destabilization mechanism is coalescence, which involves the fusion of individual droplets into larger droplets as a result of thinning and rupture of the intervening liquid film [32].

In many cases, emulsion destabilization follows a two-step process: initially, droplet size increases due to flocculation, coalescence, or Ostwald ripening; subsequently, once a critical droplet size is reached, gravitational forces become dominant, leading to the formation of phase-separated liquid layers [31]. Minimization of these destabilization phenomena can be achieved through an appropriate choice of emulsifying agents and by controlling emulsion rheology to obtain narrow droplet size distributions [33].

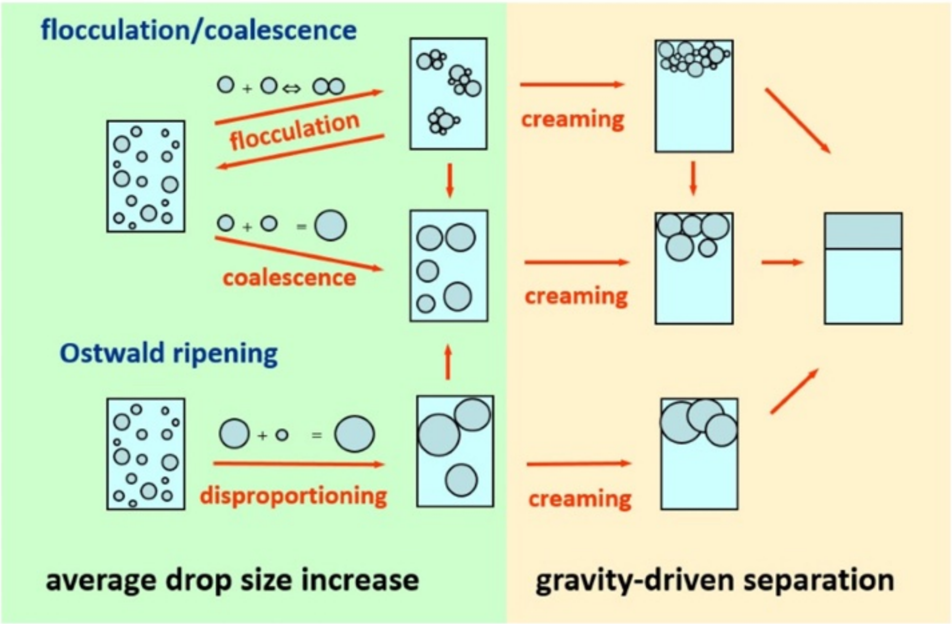


Figure 1.3.1 Schematic illustration of selected destabilization mechanisms leading to phase separation in emulsions [62].

From a formulation perspective, emulsion stability and performance are governed not only by emulsifiers, which primarily act by reducing interfacial tension, but also by additional functional ingredients. Among these, rheology modifiers, commonly referred to as thickeners, play a key role in emulsion-based cosmetic formulations. These materials may be inorganic, such as clays, fumed silica, or aluminum stearate, or organic, including polysaccharides, cellulose derivatives, and acrylic polymers. Rheology modifiers are incorporated to control viscosity, enhance suspension stability, reduce phase separation, and prevent syneresis, while simultaneously improving texture and sensory perception during product application.

Within this context, understanding the selection, optimization, and emerging trends associated with rheology modifiers is essential for the rational design of modern cosmetic emulsions, particularly in light of increasing demands for sustainable, high-performance, and sensory-optimized formulations [34].

1.4. Rheology of Pickering emulsions

Rheology is the branch of science that studies the deformation and flow characteristics of materials under the influence of external forces [35]. The flow behavior of emulsions is essential for their applications, influencing their spreadability on surfaces, sensory attributes, and long-term stability [36]. In fact, the rheological characterization of Pickering emulsions can provide valuable information about their flow characteristics and stability [37]. From an application point of view, a key rheological feature of Pickering emulsions is their pronounced thickening behavior. Elevated viscosity can effectively slow down destabilization processes, thereby enhancing emulsion stability [38]. Thickening is particularly relevant in cosmetic formulations, where it contributes not only to physical stability but also to desirable sensory attributes during application. Consequently, the viscosity of cosmetic emulsions must be carefully tuned to avoid extreme rheological behaviors, such as excessively fluid or overly rigid systems, while ensuring long-term stability under storage conditions. The stabilizing efficiency of particles in Pickering emulsions is primarily attributed to their strong adsorption at the droplet–continuous phase interface, where they form densely packed interfacial networks. These structures impart mechanical rigidity and increase interfacial viscosity to the droplets, thereby influencing the bulk rheological properties of the emulsion [39], [40].

Rheological modifiers can be divided into two main macro-classes: organic and inorganic modifiers. Organic rheological modifiers can be further categorized into three groups based on their origin. Natural modifiers are derived from plant, animal, or microbial sources and typically consist of high-molecular-weight polymers, mainly proteins or polysaccharides, such as gums [41]. Naturally modified modifiers consist of natural polymers that have undergone specific chemical modifications in order to improve their performance in the final formulation. Synthetic modifiers, on the other hand, are produced from petrochemical-based polymers. Inorganic rheological modifiers comprise exclusively mineral-based substances, including clays and pyrogenic (or fumed) silica [42].

As current demand trends push toward greener, sustainable, and non-oil-based raw ingredients, especially in the cosmetic field, the applicability of natural constituents in the rheology modifiers field is increasing. Among natural organic rheological modifiers, polysaccharides represent a particularly relevant subclass due to their wide availability, structural versatility, and ability to strongly influence both bulk and interfacial rheological properties. Owing to their high molecular weight and diverse chemical functionalities, polysaccharides can act not only as viscosity enhancers in the continuous phase but also as effective stabilizing agents in Pickering emulsions when employed in particulate form. [43]. This dual functionality allows polysaccharides to bridge the traditional distinction between rheological modifiers and emulsion stabilizers. In this context, polysaccharide-based particles have emerged as promising alternatives to conventional synthetic modifiers and inorganic stabilizers. Materials such as starch, chitosan and cellulose have been extensively investigated for their capacity to adsorb at oil–water interfaces and form mechanically robust interfacial layers, thereby imparting enhanced stability and distinctive rheological behavior to Pickering emulsions [44].The ability of these biopolymeric particles to stabilize emulsions through purely physical mechanisms, combined with their biodegradability and biocompatibility, aligns with the overarching objectives of sustainable formulation design.

2. Results

2.1. Development of a sustainable Pickering emulsion

Nowadays, the cosmetic market has increasingly moved to very simple formulations containing only essential ingredients necessary to ensure the claimed efficiency. This trend reflects growing consumer expectations regarding product safety, efficacy, and transparency, as well as a preference for shorter and more comprehensible ingredient lists [45]. Within this framework, considerable interest has been directed toward multifunctional ingredients capable of fulfilling more than one role within a formulation. Pickering emulsions, stabilized by solid particles rather than surfactants, have gained interest as multifunctional ingredients. Their particles adsorb at the droplet interface due to the partial wettability of both phases and act as effective emulsifiers without requiring a classical amphiphilic structure, making them attractive for simplified and innovative cosmetic formulations [46]. Before evaluating how modified starch, chitosan, and carboxymethylcellulose-based microparticles might perform in cosmetic formulations, a preliminary formulation study was conducted to design a model Pickering emulsion system. This base formulation was developed to meet key requirements relevant to sustainable cosmetic applications and to provide a reliable benchmark for the subsequent evaluation of particle-modified systems. In particular, the formulation strategy was guided by the selection of emulsifying agents of natural origin and fully biodegradable ingredients, in line with current trends toward environmentally friendly and clean-label cosmetic products. Special attention was devoted to minimizing the overall number of ingredients, resulting in a simplified INCI list, while ensuring adequate physical stability and functional performance. Furthermore, formulation processes were deliberately designed to be predominantly cold-based, thereby reducing energy consumption and preserving the integrity of temperature-sensitive raw materials. Vegetable oils were chosen as the dispersed phase. The choice of emulsion components and their ratios is critical for generating stable emulsion systems [47]. The formulation study was focused on the development of a sustainable oil-in- water Pickering emulsion. The emulsion composition includes:

- **Water phase**: consists primarily of purified water, which acts as the main solvent of the formulation. Due to the high-water content and the consequent susceptibility of cosmetic emulsions to microbial contamination, a suitable preservative system is included to ensure microbiological stability throughout the product's shelf life. In this context, the incorporation of a humectant such as glycerin represents a functional complement to the aqueous phase. Glycerin is widely employed for its ability to attract and retain water within the outer layers of the stratum corneum, thereby enhancing skin hydration and contributing to improved sensorial properties upon application [48]. In addition to its moisturizing function, glycerin can influence the physicochemical and rheological properties of the continuous phase, contributing to viscosity modulation and overall emulsion stability.

- **Oil phase**: composed of selected vegetable oils that are widely used in cosmetic technology due to their emollient properties, biodegradability, and compatibility with skin lipids, which contribute to moisturizing effects and a desirable sensory profile upon application. In addition to their functional roles, such oils act as carriers for oil-soluble active compounds and lipophilic ingredients, facilitating their delivery within the emulsion system [49].
- **Emulsifiers**: in Pickering emulsions, solid particles irreversibly adsorbed at the oil–water interface stabilize the dispersion of oil droplets and prevent coalescence and phase separation. Because these particles do not require the amphiphilic molecular structure characteristic of conventional surfactants, they can fulfil the role of emulsifier while contributing to the formulation's biodegradability and reduced reliance on synthetically derived surfactants [50].

2.2. Ingredients screening and selection

As an initial formulation strategy, two natural fibers derived from agri-food by-products, apple fiber (Vitacel CS 5) and citrus fiber (Vitacel CF 312), were selected and evaluated as particulate emulsifiers. The choice of these materials was guided by multiple considerations: their natural origin, biodegradability, and alignment with sustainable formulation principles, as well as their availability at the host laboratory (AgroParisTech, Orléans, France). In addition, both fibers are rich in polysaccharidic components (mainly cellulose, hemicellulose, and pectin), which are known to promote water binding, viscosity enhancement, and interfacial stabilization, making them promising candidates for Pickering emulsions [51], [52]. In this preliminary screening, the formulations were intentionally simplified to assess the intrinsic emulsifying ability of the fibers. The ingredient list was therefore limited to water as the continuous phase, glycerin as a humectant, a vegetable oil phase, and the fibrous particles as the sole stabilizing agents, without the addition of conventional surfactants. When a combination of apple and citrus fibers was employed, the resulting emulsions exhibited satisfactory physical stability over time, indicating effective droplet stabilization. However, the systems displayed a distinct brownish coloration (**Table 2, Test 1**), which is attributable to the natural pigments and phenolic compounds present in apple fiber. Although structurally stable, this aesthetic characteristic was considered unsuitable for conventional cosmetic creams, limiting potential applications to tinted formulations. Conversely, formulations stabilized exclusively with citrus fiber resulted in inhomogeneous, paste-like systems that showed poor structural organization and limited stability (**Table 2, Test 2**). This behavior may be related to the high water-binding capacity and irregular particle morphology of citrus fiber, which can hinder uniform droplet coverage and lead to weak interfacial packing [53]. Overall, while both fibers demonstrated some degree of emulsifying capability, the observed limitations in color and microstructural homogeneity highlighted the need to identify alternative stabilizers offering improved functional performance and sensory acceptability. Based on the outcomes of the initial screening, a different natural-derived emulsifier, Vivapur CS TEX ESY, was subsequently selected. Vivapur is a commercially available stabilizing system composed of microcrystalline cellulose, cellulose gum, and xanthan gum, and is widely used in cosmetic and food emulsions. Its white color, high purity, and well-documented stabilizing efficiency made it a suitable candidate for the development of a neutral and aesthetically acceptable Pickering base emulsion [54]. Given its white appearance, Vivapur was also tested in combination with apple fiber in an attempt to mitigate the intense coloration associated with the latter while preserving its advantageous properties, such as sustainability, natural origin, and structural contribution. However, even in this blended system, the resulting emulsions retained a light brown coloration. These formulations could nonetheless be considered for colored products, such as BB creams or tinted emulsions, where pigmentation is desirable (**Table 2, Test 3**). Vivapur CS TEX ESY was selected as the sole emulsifying and rheology-modifying agent for the development of the base Pickering emulsion. This choice was guided by several key features that make Vivapur particularly suitable for sustainable cosmetic formulations, including its high natural origin content (98.9% wood-derived polysaccharides), biodegradability, white color, and multifunctional composition. Vivapur is a synergistic blend of microcrystalline cellulose, cellulose gum, and xanthan gum, enabling emulsion stabilization both through particulate adsorption and viscosity enhancement of the continuous phase.

2.3. Development of the base emulsion

A wide range of combinations was investigated, leading to the first formulation (**Table 2, Test 4**) incorporating Vivapur. The formula was prepared using sunflower oil and plum oil as the dispersed lipid phase. In line with the sustainability-driven formulation strategy adopted in this work, the ingredient list was deliberately kept to a minimum and included only purified water as the main solvent, a preservative (Potassium sorbate) to ensure microbiological stability, and glycerin as a humectant. The resulting emulsion exhibited a homogeneous white appearance, which is highly desirable for cosmetic applications, together with good macroscopic stability and suitable rheological properties. Although the formulation showed satisfactory physical stability, minor limitations related to texture and sensorial perception were observed, indicating that further refinement was required. Nevertheless, this Vivapur-based system was considered sufficiently robust and reproducible to be selected as a model formulation, serving as the starting point for a systematic optimization study.

Trials	Emulsifiers	Observations	Images
Test 1	 Vitacel CS 5 Apple Fiber  Vitacel CF 312 Citrus Fiber	Physical stability over time, but brownish coloration	
Test 2	 Vitacel CF 312 Citrus Fiber	Inhomogeneous, paste-like system, with limited stability	
Test 3	 Vivapur CS TEX Easy  Vitacel CS 5 Apple Fiber	Physical stability over time,but light brownish coloration	
Test 4	 Vivapur CS TEX Easy	Physical stability over time,white coloration	

Table 2. Comparative evaluation of natural powders employed as particulate stabilizers in Pickering emulsions, including formulation observations and representative photographs of the corresponding formulations.

2.4. Design of experiments and stability assessment of Vivapur-based Pickering emulsions

To systematically investigate the influence of formulation variables on the stability of the base Vivapur stabilized Pickering emulsion, a Design of Experiments (DoE) approach was implemented using the software JMP. The DoE was structured as a response surface design, with two independent variables: Vivapur concentration (3–8 wt %) and total oil content (10–40 wt %, comprising sunflower and plum oils). The primary objective was to identify the minimal effective concentrations of both the solid stabilizer and oils capable of producing a physically stable emulsion. Ten experimental formulations were generated by the DoE and prepared under standardized emulsification conditions. This approach offers several advantages: it enables systematic evaluation of ingredients interactions, minimizes the number of experimental trials needed, and provides predictive models that can be used to optimize formulation parameters for targeted stability and droplet characteristics [55].

The physical stability of the emulsions was assessed using a Turbiscan Lab, an analytical instrument that measures backscattered (BS) and transmitted light along the height of the sample over time. The Turbiscan detects droplet migration, creaming, sedimentation, and coalescence with high sensitivity, providing a quantitative profile of emulsion destabilization without the need for dilution or sample perturbation [56], [57]. For each sample, the initial backscattering value (BS_0) in the range of 5–20 mm from the base of the cell was extracted and used as the primary parameter for analysis. BS_0 is particularly informative because it correlates with droplet size: higher BS_0 values indicate smaller droplets and higher initial stability. Based on these data, two indices were calculated:

- **Creaming index**: quantifies the upward migration of droplets (creaming) without fusion.
- **Coalescence index**: measures the fusion of droplets, resulting in larger droplets and loss of stability [58].

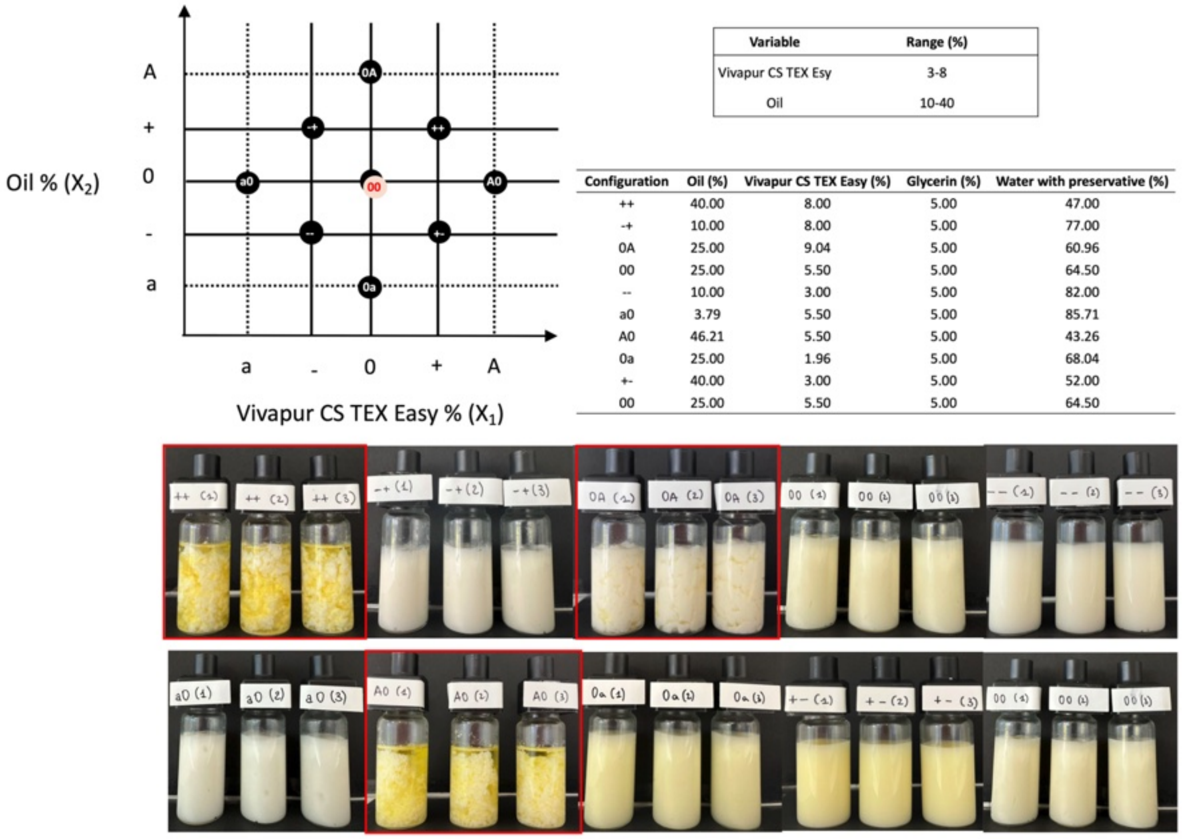
Additionally, the change in backscattering over time ($\Delta BS = BS_0 - BS\_days$) and the change in emulsion height ($\Delta H = H_0 - H\_7days$) were evaluated to monitor destabilization kinetics.

The experimental data were input into JMP for predictive modeling. Analysis revealed that the total oil content was the most influential variable affecting BS_0, with a quadratic (parabolic) relationship. This indicates that emulsion stability, in terms of droplet size distribution, is highly sensitive to the amount of dispersed phase: both very low and very high oil concentrations produce distinct effects, which can reverse under certain conditions (crossover effect). Vivapur concentration also showed a significant impact on BS_0, although to a lesser extent, and its effect was non-linear. The observed crossover behavior between oil and Vivapur highlights a dual interaction, emphasizing the value of a DoE approach for capturing complex formulation dependencies.

Regarding the creaming index, most experimental formulations exhibited very low values (<1%), indicating minimal upward migration of droplets. Due to the lack of variability among the samples, this parameter was not useful for predictive modeling. Similarly, changes in emulsion height were negligible and, therefore, were not considered in the modeling process.

The coalescence index remained low across the experimental domain, suggesting that most tested formulations were generally resistant to droplet fusion, a desirable outcome for long-term stability. However, this limited variation also reduced the sensitivity of the DoE model in predicting coalescence behavior. The predictive modeling of BS_0 provides valuable insights into the formulation design of Vivapur-stabilized Pickering emulsions. By adjusting the oil content while maintaining a fixed concentration of Vivapur, it is possible to modulate droplet size, optimize viscosity, and tailor the rheological and sensorial properties of the emulsion. Conversely, fine-tuning Vivapur levels allows additional control over interfacial rigidity and droplet stabilization, although with a smaller effect than the oil concentration. This approach thus enabled both rational selection of formulation parameters and the construction of a predictive framework to guide subsequent optimization, reducing experimental workload while improving understanding of the relationship between composition, microstructure, and stability.

The ten experimental formulations generated by the DoE were prepared using the standardized emulsification protocol under controlled storage conditions (4 °C, 25 °C, and 45 °C) and subsequently evaluated for macroscopic stability. As shown in **Figure 2.4.1**, visual inspection revealed that three formulations (++, 0A, and A0) exhibited clear signs of instability, already indicating unfavorable combinations of the tested variables. Specifically, formulation ++ showed significant phase separation, with aggregates, as well as high heterogeneity. Significant flocculation was shown in formulation 0A, with large domains of dispersed oil drops, indicating insufficient structuring in the stabilization process for the liquid phase. Formulation A0 showed a visible yellowing on its top portion because of oil separation and coalescence.



Variable	Range (%)
Vivapur CS TEX Esy	3-8
Oil	10-40

Configuration	Oil (%)	Vivapur CS TEX Easy (%)	Glycerin (%)	Water with preservative (%)
++	40.00	8.00	5.00	47.00
-+	10.00	8.00	5.00	77.00
0A	25.00	9.04	5.00	60.96
00	25.00	5.50	5.00	64.50
--	10.00	3.00	5.00	82.00
a0	3.79	5.50	5.00	85.71
A0	46.21	5.50	5.00	43.26
0a	25.00	1.96	5.00	68.04
+-	40.00	3.00	5.00	52.00
00	25.00	5.50	5.00	64.50

Figure 2.4.1. Representative images of samples generated through the Design of Experiments (DoE). Red boxes denote conditions leading to the formation of visible yellow aggregates or precipitates.

From these observations, it can be determined that formulations ++, 0A, and A0 acted as true outliers within the experimental design, with clear visual instability even from the early evaluation. These particular variable combinations led to poor emulsification and poor kinetic stability, as was afterwards confirmed by the Turbiscan stability analysis. The remaining formulations were subsequently subjected to an accelerated stability test. Approximately 10 mL of each emulsion was centrifuged at 4000 rpm for 30 minutes at room temperature to evaluate their propensity for phase separation under stress conditions. The results indicated that formulations +- and 0a exhibited signs of long-term instability, including visible phase separation, and were therefore excluded from further investigation (**Figure 2.4.2**).

Only the four remaining formulations (00, --, a0 and -+) were selected for reformulation and more detailed characterization. The stability of these selected emulsions was further assessed at time zero and after 7 days under three different storage temperatures (4 °C, 25 °C, and 45 °C) using the Turbiscan**.**

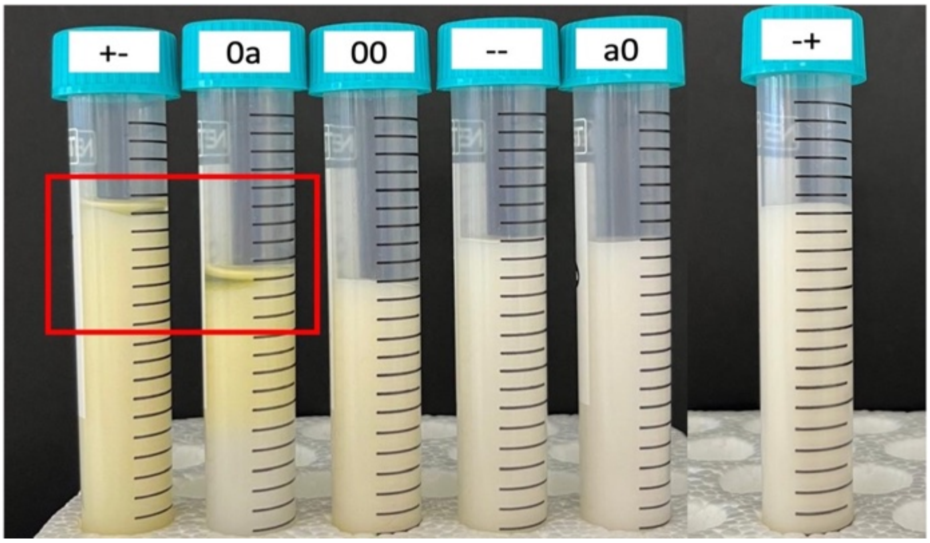


Figure 2.4.2 Photographs of the emulsions after accelerated stability testing by centrifugation. The image illustrates the phase behavior of each formulation under stress conditions, highlighting the presence of phase separation in formulations +- and 0a.

This allowed the evaluation of both macroscopic and sub-microscopic destabilization processes, including creaming and coalescence. Following the Turbiscan analysis, the emulsions were again subjected to an accelerated stability assessment**,** which revealed the presence of a thin layer of separation in formulations 00 and --. (**Figure 2.4.3**). These minor destabilization events confirmed that, among the initially selected candidates, only two formulations demonstrated robust stability under both standard and stressed conditions.

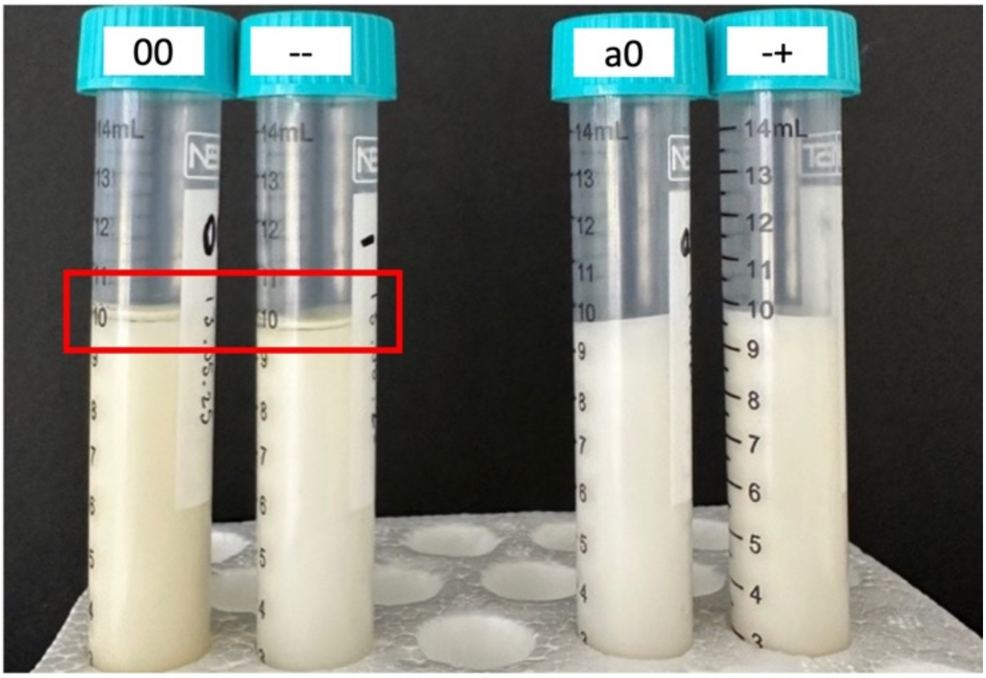


Figure 2.4.3. Photographs of the emulsions after accelerated stability testing by centrifugation. The image illustrates the phase behavior of each formulation under stress conditions, highlighting the presence of a thin layer of separation in formulations 00 and --.

Among these two stable emulsions, formulation a0 was chosen as the model base for the incorporation of microparticles for several reasons: firstly, it demonstrated superior physical stability, secondly, it contained the lowest amounts of oil and emulsifier, aligning with the goal of minimalistic, sustainable formulations; thirdly, its white coloration was advantageous for cosmetic applications; and finally, its sensory properties, particularly spreadability and skin feel, were preferable compared to the alternative stable formulation.

2.5. Characterization of a0 base emulsion

The selected formulation a0 was further characterized in terms of its rheological behavior, droplet size distribution, and organoleptic properties, in order to assess its suitability as a stable cosmetic Pickering emulsion. Rheological measurements revealed that formulation a0 exhibits a shear-thinning (pseudoplastic) behavior, which is characteristic of non-Newtonian fluids. Specifically, the apparent viscosity decreased progressively with increasing shear rate, indicating that the emulsion becomes less resistant to flow when subjected to mechanical stress, such as stirring or spreading. Upon removal of the applied shear, the formulation recovered its initial viscosity, reflecting the reversible nature of the internal microstructure [59], [60]. This rheological behavior is particularly desirable for cosmetic applications, as it facilitates ease of application while maintaining sufficient viscosity at rest to prevent phase separation. Oscillatory strain sweep measurements were performed to investigate the viscoelastic properties and structural integrity of the emulsion. In this test, an increasing deformation (strain, %) was applied to determine the extent of the linear viscoelastic region (LVR), within which the internal structure of the material remains intact. At low strain values (up to approximately 10%), the storage modulus (G′) was significantly higher than the loss modulus (G″), indicating that the emulsion behaves predominantly as an elastic solid, capable of storing mechanical energy due to the presence of a well-developed internal network.

As the applied strain increased, a crossover point between G′ and G″ was observed at 64.3 % approximately. This crossover marks the transition from a predominantly elastic response (G′ > G″) to a viscous-dominated behavior (G′ < G″), corresponding to the disruption and collapse of the emulsion's internal structure. The relatively high crossover strain value indicates a good mechanical resistance of the formulation, as it can withstand substantial deformation before structural breakdown occurs. Overall, formulation a0 displays a dominant solid-like behavior within the linear viscoelastic region, consistent with a structurally robust Pickering emulsion (**Figure 2.5.1**)

Particle size analysis revealed an average droplet diameter of approximately 23 µm. The pH value was measured at 5.6, which is compatible with the physiological pH of human skin and therefore suitable for topical cosmetic applications. The formulation exhibited a white coloration and a creamy texture, which were perceived as pleasant upon tactile evaluation, further supporting its selection as a model system for subsequent experiments involving the incorporation of natural and modified particulate stabilizers.

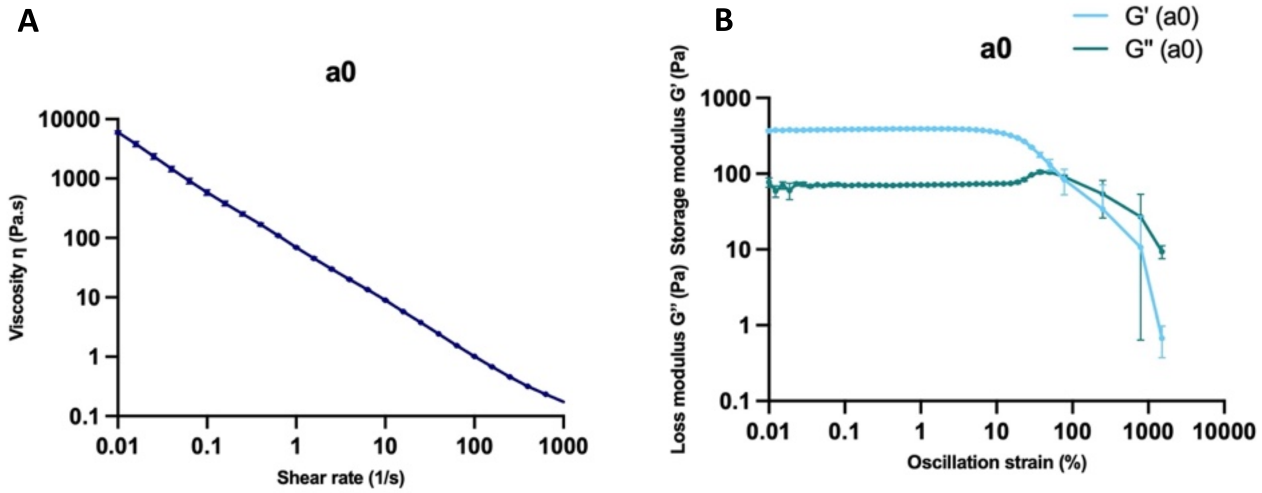


Figure 2.5.1 Rheological characterization of the formulations. (a) Flow sweep curve showing the shear-dependent viscosity and flow behavior. (b) Strain sweep profiles evidencing the viscoelastic region and structural integrity of the sample.

2.6. Incorporation of sustainable microparticles into the Pickering emulsion system

Following the development of the optimized sustainable Pickering base emulsion, preliminary incorporation trials were conducted to evaluate the feasibility of using the synthesized biodegradable microparticles as stabilizing or co-stabilizing agents. Given the objective of replacing microplastics in cosmetic formulations, particular attention was placed on determining whether microparticles derived from modified starch, chitosan or carboxymethylcellulose could contribute to emulsion stabilization while maintaining acceptable sensorial and physicochemical properties. The first part of experiments was conducted using starch-based microparticles. This choice was primarily driven by practical considerations related to material availability: compared to chitosan- and carboxymethylcellulose-based microparticles, starch microparticles were available in larger quantities, allowing a more extensive and systematic preliminary evaluation. Although, to limit the use of modified powders during early formulation trials, the first series of experiments employed native tapioca starch. Native starch was dispersed and gelatinized prior to incorporation into the aqueous phase. However, an emulsion prepared using native starch as the sole stabilizing agent exhibited clear instability and rapid phase separation, confirming that native starch alone is insufficient to stabilize the oil–water interface in this system.

To overcome this limitation, a combination of native starch and Vivapur was tested (**Figure 2.6.1**, **F1**). This formulation displayed markedly improved stability and a homogeneous creamy texture, demonstrating that starch-based microparticles can function effectively only when used in synergy with a primary stabilizing agent. This finding guided the subsequent formulation strategy.

Building on the insights obtained with native starch, three additional formulations (**Figure 2.6.1, F2–F4**) were prepared incorporating modified starch microparticles alongside Vivapur. Two types of modifications were investigated: oleic acid-modified starch (**F2**) and PMW-modified starch (**F3**, **F4**). The incorporation levels were selected based on preliminary dispersibility and compatibility tests, and the corresponding concentrations are detailed in the *Materials and Methods section*. Formulation F0 corresponds to formulation a0, which was selected as the model Pickering emulsion stabilized exclusively by Vivapur and used as the reference system for all subsequent experiments involving the incorporation of biopolymer-based microparticles.

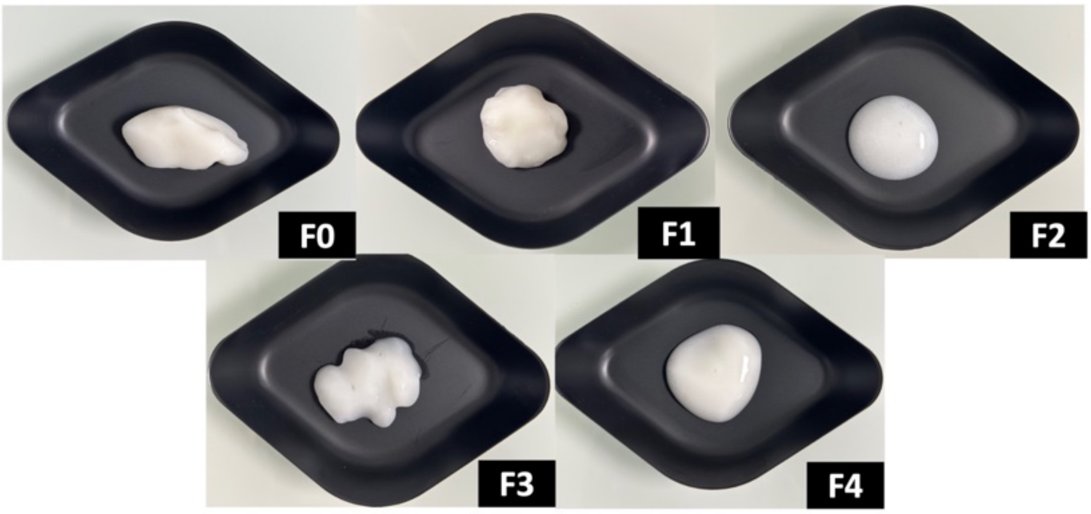


Figure 2.6.1 Photographic images of the obtained formulations, illustrating their macroscopic appearance.

All starch-containing formulations exhibited pH values between 5.5 and 5.8, suitable for topical applications. According to visual inspection and Turbiscan stability profiling, formulations containing modified starches remained physically stable at time 0 and after 7 days of storage. Accelerated stability testing under centrifugation corroborated the absence of phase separation, confirming that modified starches do not negatively affect the emulsion's structural integrity under stress conditions (**Figure 2.6.2**).

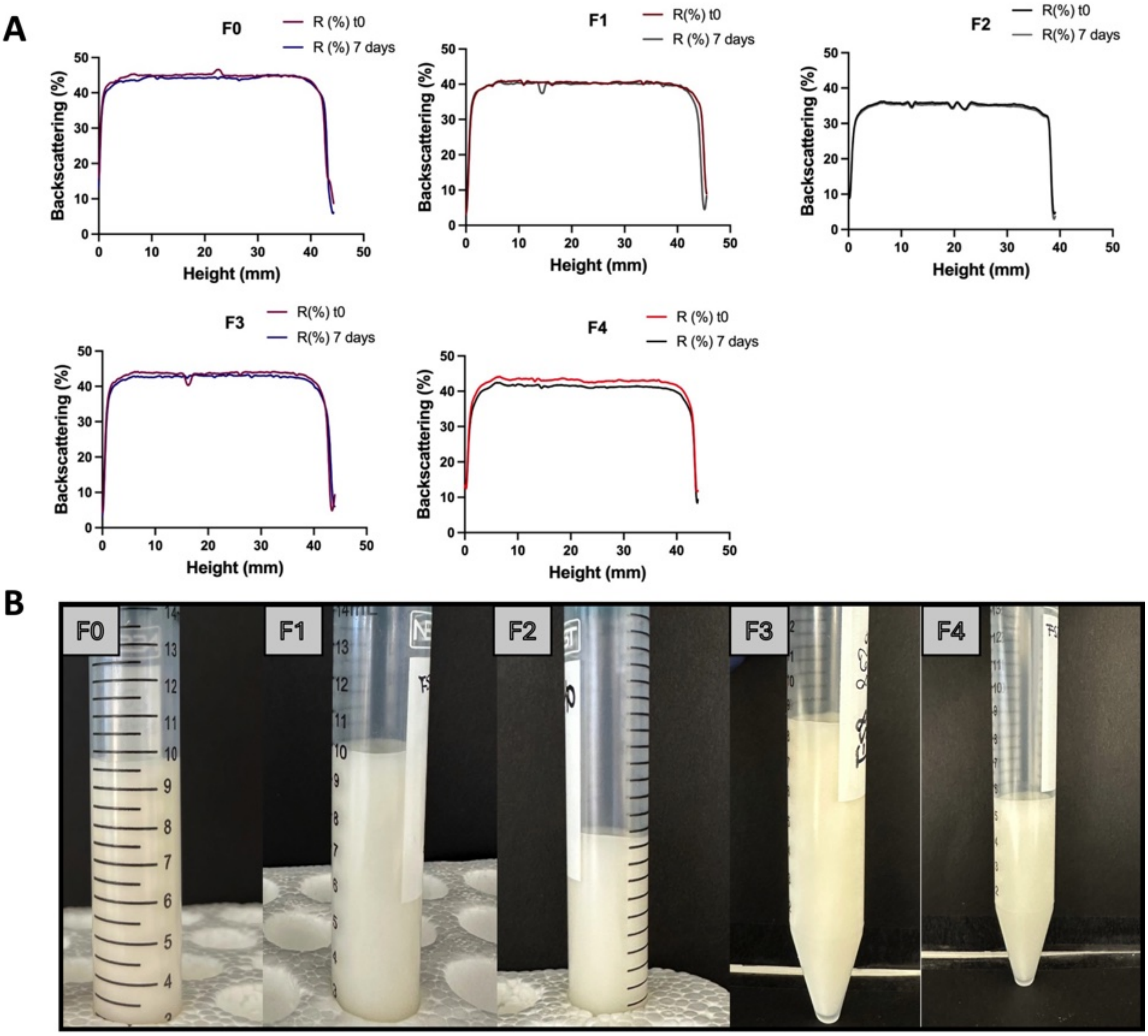


Figure 2.6.2 Emulsion stability of formulations F0–F4 evaluated by (a) Turbiscan analysis at initial (t_o) and 7-day time points, and (b) visual inspection after accelerated centrifugation.

The rheology of formulations is an important quality parameter. In this study, the apparent viscosity of the starch-based emulsions decreased with the increase of shear rate from 0.1–100 s− 1 as shown in **Figure 2.6.3** (a), indicating that the emulsions demonstrate a shear-thinning and non-Newtonian fluid behavior. The shear-thinning behavior is a desirable feature for topical formulations as it facilitates the application of the product on the skin and leaves a smooth sensorial feeling.

Formulations F1, F3, and F4 exhibited crossover values around 90%, indicative of a robust viscoelastic network, while F2, with oleic acid-modified starch, displayed a lower crossover value (63%), suggesting a reduced elastic contribution and earlier structural breakdown under strain.

The strength of the network structure can be determined from the storage modulus (G′) which indicates the elastic properties of the emulsion, and the loss modulus (G′′) that indicates the viscous properties of the emulsion. The results of the dynamic oscillatory measurements of the Pickering emulsions with the frequency ranging from 0.01–10 Hz are shown in **Figure 2.6.3** (b). Vivapur alone produced the most rigid network (highest G’), followed by Vivapur combined with PMW-modified starch, and finally the native or oleic acid-modified tapioca starch formulations. This trend reflects the reinforcing effect of the PMW-modified particles and the softer contribution of oleic acid-m modified starches

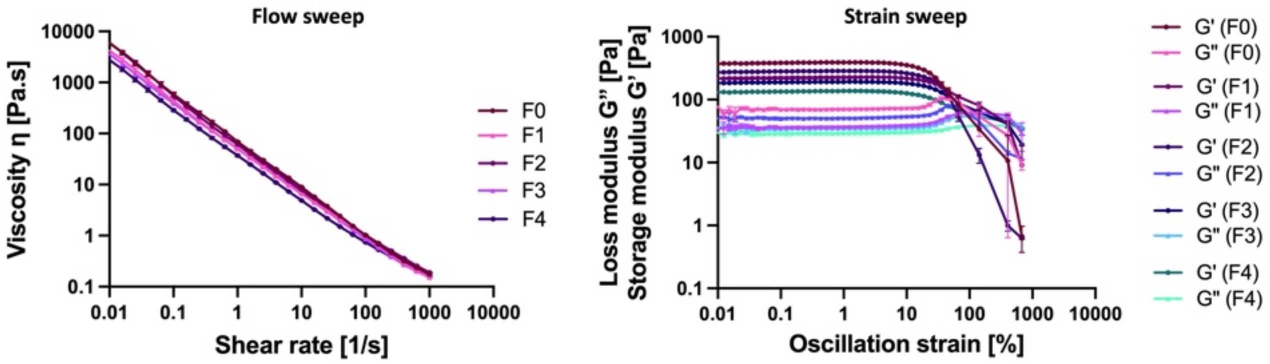
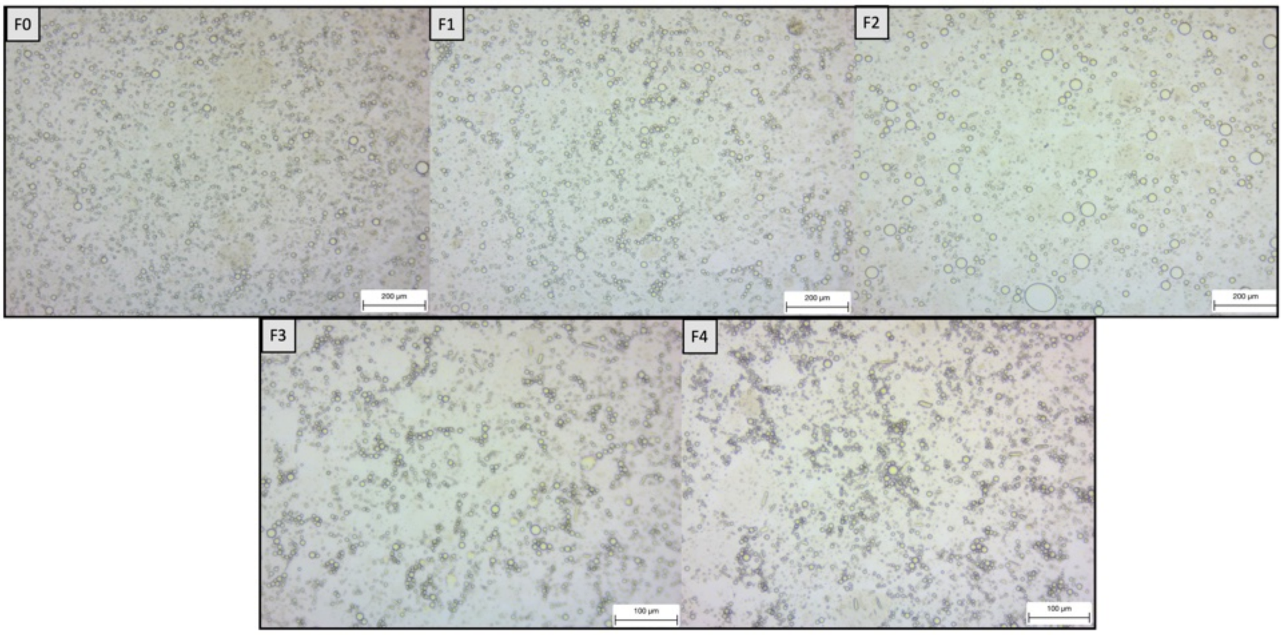


Figure 2.6.3 Rheological characterization of the formulations. (a) Flow sweep curves showing the shear-dependent viscosity and flow behavior. (b) Strain sweep profiles evidencing the viscoelastic region and structural integrity of the samples.

Droplet size distribution analysis further confirmed the strong influence of particle type on emulsion microstructure. The base emulsion (F0) exhibited a reduced mean droplet size and a more homogeneous distribution. In contrast, the incorporation of native starch or oleic acid–modified starch led to an increase in average droplet diameter. Among the tested systems, emulsions containing tapioca starch PMW (F3–F4) displayed the narrowest droplet size distribution, indicating enhanced droplet stabilization and improved uniformity. (**Figure 2.6.4**).



	Median D (µm)	Modal D (µm)	Mean V (µm)	Std Dev (µm)
F0	14.02	12.34	14.34	3.30
F1	16.27	11.77	17.35	5.83
F2	17.56	26.90	20.00	8.37
F3	6.70	7.11	6.69	1.38
F4	7.17	6.77	7.27	2.53

Figure 2.6.4 Representative optical micrographs of the emulsions with associated particle size statistics (median, mode, mean, and standard deviation) obtained through granulometric analysis.

Overall, these results indicate that starch-based microparticles, particularly modified variants, are compatible with the optimized Pickering emulsion matrix and can be successfully incorporated without compromising stability or rheological behavior. Their influence on droplet size and viscoelasticity suggests potential as co-stabilizers, supporting their suitability as sustainable alternatives to microplastics.

Two formulations (F5 and F6) were prepared using oleic acid–modified chitosan microparticles, either as the sole particulate stabilizer or in combination with Vivapur. In contrast to the starch-based systems, these emulsions exhibited highly viscous, sticky, and pudding-like textures, which were not compatible with the rheological and sensorial requirements of cosmetic formulations. The lack of structural cohesion and poor spreadability significantly limited their practical applicability. (**Table 3**).

Furthermore, stability tests revealed discoloration upon storage at elevated temperature (45 °C), suggesting chemical or physical instability and a reduced robustness of the system when compared with starch-containing analogues. These findings indicate that, under the formulation conditions investigated, chitosan-based microparticles do not provide effective stabilization of the Pickering emulsion and may interact unfavorably with the aqueous polymeric network formed by Vivapur, potentially leading to excessive network densification or phase incompatibility.

A similar approach was applied to oleic acid–modified carboxymethylcellulose (CMC) microparticles, which were evaluated in combination with Vivapur. However, the resulting formulation exhibited clear physical instability, further confirming the limitations of these systems within the current formulation design.

Rheological characterization of chitosan-based formulations supported these observations, revealing undesirable viscoelastic profiles associated with overly rigid textures. Although these microparticles appeared particularly promising from a morphological point of view, as their spherical shape closely resembles that of conventional microplastic particles, their macroscopic formulation behavior did not translate into functional or sensorially acceptable emulsions.

Table 3. Comparative evaluation of chitosan-based emulsions natural powders employed, including formulation observations and representative photographs of the corresponding formulations.

Trial	Composition/Ratio	Process condition	Observation	Image
F5	Chitosan oleic acid + Vivapur (1:1)	Cold process	Sticky film-forming texture; yellowish	
F6	100% chitosan oleic acid	Cold process	Sticky texture; yellowish	

These results suggest that, despite their favorable particle geometry and theoretical suitability as Pickering stabilizers, oleic acid–modified chitosan and CMC microparticles require further optimization. Parameters such as, particle concentration, emulsification conditions, or the ratio between particulate stabilizers and polymeric co-stabilizers should be systematically explored.

3. Discussion

This study aimed to evaluate the ability of the developed biopolymer microparticles to act as Pickering stabilizers in oil–water emulsions. The selection of formulation parameters, including particle concentration, oil phase composition, and homogenization conditions, was based on their known influence on particle adsorption at the oil–water interface and emulsion stability. Particle concentration was varied to identify the minimum amount required to ensure full interfacial coverage and prevent droplet coalescence. Oil phase composition was selected to evaluate the compatibility between the modified particle surface and the dispersed phase, while homogenization conditions were optimized to control droplet size distribution. The improved stability observed for modified particles can be attributed to their increased surface hydrophobicity, which enhances adsorption at the interface and promotes the formation of a rigid particle layer around droplets. The present study demonstrates a rational formulation strategy for developing a sustainable Pickering emulsion suitable for cosmetic applications, combining minimal ingredient complexity, biodegradability, and robust physicochemical performance. In particular, the objective was to evaluate the ability of bio-based particles to act as effective Pickering stabilizers and to identify formulation parameters that govern emulsion stability, microstructure, and rheological behavior. The initial screening of natural fibrous materials highlighted the intrinsic challenges associated with the direct use of agri-food by-products as particulate emulsifiers. Although apple and citrus fibers exhibited partial emulsifying capability, their strong coloration, irregular microstructure, and limited homogeneity compromised aesthetic acceptability and formulation reproducibility. These findings underline the importance of balancing sustainability-driven material selection with sensory and technological constraints that are critical in cosmetic products. The selection of Vivapur as the primary stabilizing system was based on its dual functionality as both a particulate stabilizer and a structuring agent. Its composition, combining microcrystalline cellulose, cellulose gum, and xanthan gum, enables not only adsorption at the oil–water interface but also viscosity enhancement of the continuous phase, which is known to reduce droplet mobility and coalescence. This multifunctional behavior is particularly advantageous in Pickering systems, where both interfacial coverage and bulk rheology contribute to overall stability. As a result, homogeneous, white emulsions with satisfactory rheological properties were obtained. The implementation of a Design of Experiments (DoE) approach provided a quantitative and predictive framework to systematically investigate the combined effects of key formulation variables. In particular, oil content and particle concentration were selected as critical parameters due to their direct influence on droplet size, interfacial coverage, and network formation. This approach allowed the identification of optimal conditions while minimizing the number of experimental trials, ensuring a rational and efficient formulation strategy. The strong, non-linear dependence of the backscattering signal on oil fraction confirms that droplet size and microstructure are primarily governed by dispersed phase loading, while Vivapur concentration exerts a secondary but significant modulatory role on interfacial rigidity and bulk viscosity. Among the tested conditions, formulation a0 emerged as the optimal compromise between stability, low ingredient load, aesthetic neutrality, and favorable sensorial attributes. Its shear-thinning behavior and dominant elastic response within the linear viscoelastic region are consistent with a percolated droplet–particle network, which confers mechanical resistance while maintaining good spreadability during application. The relatively high crossover strain indicates that the internal structure can tolerate substantial deformation before breakdown, a desirable feature for handling, packaging, and consumer use. The measured droplet size and skin-compatible pH further support its suitability as a cosmetic base system. The incorporation of biodegradable starch-based microparticles demonstrated that these materials can act as effective co-stabilizers when combined with Vivapur. Native starch alone was insufficient to stabilize the oil–water interface, confirming that surface chemistry and wettability are critical determinants of Pickering performance. In Pickering emulsions, particles must exhibit partial wettability toward both phases to irreversibly adsorb at the interface and form a protective barrier against coalescence. In contrast, modified starches, particularly PMW-modified variants, enhanced droplet uniformity and contributed positively to the viscoelastic network, as evidenced by narrower size distributions and higher storage moduli compared to native or oleic acid–modified starch systems. These results suggest that controlled chemical modification improves interfacial affinity and particle–matrix interactions, enabling better packing at the droplet surface and reinforcing the three-dimensional microstructure. Importantly, the maintenance of shear-thinning behavior across all starch-containing formulations indicates that functional performance and sensory applicability are preserved. Conversely, oleic acid–modified chitosan and carboxymethylcellulose microparticles exhibited poor formulation compatibility, leading to excessive viscosity, sticky textures, discoloration under thermal stress, and, in some cases, physical instability. These effects likely arise from strong polymer–polymer interactions and over-densification of the aqueous network, which may hinder optimal particle adsorption at the oil–water interface and compromise microstructural balance. While their spherical morphology is attractive as a microplastic substitute, these systems require further optimization in terms of particle concentration, surface modification degree, and emulsification protocol to achieve acceptable cosmetic performance.

Overall, this work confirms that sustainable Pickering emulsions can be engineered through a combination of multifunctional biopolymeric stabilizers and rational experimental design. Compared to conventional surfactant-based systems, this approach offers the advantage of reduced synthetic ingredient content while maintaining stability and tunable rheology. Modified starch microparticles emerge as promising candidates for partial replacement of synthetic microplastics, offering compatibility with green formulation principles while maintaining stability and tunable rheology. Future studies should focus on scalability, sensory panel evaluation, and the extension of this approach to active ingredient delivery and industrial processing conditions, in order to fully validate the translational potential of these systems for commercial cosmetic applications.

4. Materials and Methods

4.1 Reagents

Tapioca starch was purchased from Nouryon Chemicals SPA (Milan, Italy). Chitosan low molecular weight, Carboxymethylcellulose sodium salt and Potassium sorbate were purchased from Sigma-Aldrich (St. Louis, MI, USA). Sunflower oil (commercial food grade Fruit d'Or) was acquired from a local retail market (Orléans, France) and used as received without further purification.
The Glycerin and Plum oil were obtained from Aroma-zone (Paris, France). VIVAPUR® CS TEX Easy, Vitacel CS 5, Vitacel CF 312 were generously supplied by JRS Retteinmaier (Rosenberg, Germany).

4.2. Cream formulation

Oil-in-water (O/W) cream emulsions with a semisolid consistency were formulated in this study. The dispersed phase consisted of a blend of vegetable oils (sunflower oil and plum oil), selected for their emollient properties, biodegradability, and suitability for cosmetic applications. The continuous aqueous phase was composed of purified water as the main solvent, glycerin as a humectant, and potassium sorbate as a preservative to ensure microbiological stability of the formulations. Emulsification was achieved using solid particles emulsifiers, according to the Pickering emulsion concept. Pickering stabilizers of different chemical nature and origin were investigated in this work. Depending on their specific physicochemical properties, such as wettability and affinity for the oil or aqueous phase, the solid particles were incorporated either into the aqueous phase or into the oil phase prior to emulsification.

4.3. Development of a Pickering base emulsion

Ingredient screening and selection

After defining the composition of the oil and aqueous phases, a formulation screening was carried out to develop a stable Pickering base emulsion. In this step, several solid powders of natural origin were evaluated as potential stabilizing agents. The selection of these powders was guided by sustainability criteria, cosmetic compatibility, and their potential ability to adsorb at the oil–water interface. Different types of powders were tested, and their concentrations were varied in order to assess their influence on emulsion formation, stability, and sensorial properties. Apple fiber and citrus fiber were selected as initial candidates and they were evaluated both individually and in combination as Pickering stabilizers, followed by the evaluation of Vivapur CS TEX Easy (a microcrystalline cellulose-based natural stabilizer), which was ultimately selected as the emulsifier for the base emulsion. The compositions of the tested formulations, including the type and percentage of particulate emulsifier used, are summarized in **Table 4**.

Table 4. Ingredients percentage composition (% w/w).

Ingredients	Test 1	Test 2	Test 3	Test 4
Aqueous phase				
Water	73.88	73.88	74.88	74.88
Glycerin	5	5	5	5
Potassium sorbate	0.2	0.2	0.2	0.2
Vivapur CS TEX Easy			3.00	4.00
Oil phase				
Sunflower oil	8.17	10.00	10.50	10.50
Plum oil	4.08	5.00	5.50	5.50
Vitacel CS 5	6.00		1.00	
Vitacel CF 312	2.67	6.00		

Emulsion Preparation

The base emulsion was prepared using a cold emulsification process. A blend of vegetable oils (sunflower oil and plum oil) was used as the dispersed (oil) phase. The Pickering stabilizer, VIVAPUR® CS TEX EASY, was first dispersed and pre-activated in purified water under mechanical stirring at 2000 rpm with Hei-TORQUE Core mechanical stirrer (Heidolf, Schwabach, Germany) for 5 minutes. After a resting period of 15 min to allow complete hydration and stabilization of the dispersion, glycerin was incorporated into the aqueous phase as a humectant, followed by the addition of potassium sorbate as a preservative. The resulting aqueous phase was then gradually added to the oil phase, and emulsification was performed using a high-shear homogenizer (POLYTRON® PT 10-35 GT, Kinematica AG, Switzerland) at 10,000 rpm for 3 min. The resulting emulsion was collected in appropriate containers and stored at room temperature until further analysis.

Optimization of base emulsion using an experimental design approach

Following the initial preparation of the base emulsion, an optimization study was conducted to determine the optimal composition of ingredients for achieving a stable formulation. A design of experiments (DoE) approach was employed using JMP software. Response Surface Methodology (RSM) was selected as the most suitable strategy, as it enables the modeling of continuous responses as functions of multiple input variables, allowing for the assessment of both individual and interaction effects.

The independent variables (X) were:

- Oil content, ranging from 10 % to 40 %, using a combination of sunflower and plum oils in a fixed 1:2 ratio.
- Vivapur content, ranging from 3 % to 8% as the stabilizing agent.

The response variable (Y) was defined as the emulsion stability, which was quantitatively evaluated using a Turbiscan optical analyzer. The experimental design consisted of one level and two factors, generating 10 configurations. Every configuration was prepared and analyzed in triplicate using the Turbiscan to ensure the robustness and reproducibility of the results.

Development of the optimized emulsion

Based on the Design of Experiment (DoE), the formulation (a0) corresponding to the highest stability was selected as the optimized base emulsion. The detailed composition, including the exact quantities and percentages of each component, is summarized in **Table 5**, and the emulsion was prepared according to the protocol described in Section *Emulsion Preparation* and used as the reference system for subsequent incorporation of microparticles.

Table 5. Ingredients percentage composition (% w/w).

Ingredients	INCI	Function	(%)
Aqueous phase			
Water	Aqua	Main solvent	85.51
Glycerin	Glycerin	Humectant	5
Potassium sorbate	Potassium Sorbate	Preservative	0.2
Vivapur CS TEX Easy	Microcrystalline Cellulose, Cellulose Gum e Xanthan Gum	Thickener,rheology stabilizer	5.50
Oil phase			
Sunflower oil	Helianthus Annuus Seed Oil	Emollient	2.53
Plum oil	Prunus Domestica Seed Oil	Emollient	1.26

4.4. Emulsion formulation incorporating natural based microparticles

Emulsion preparation with starch-based microparticles

Tapioca starch powder (native or modified) was first dispersed in distilled water and subjected to controlled heating in an oil bath at 90 °C for 1 h under mechanical stirring at 300 rpm to promote gelatinization. The resulting suspension was then allowed to cool to room temperature before incorporation into the aqueous phase of the emulsion.

After dispersion of the starch solutions, the suspensions were added to the remaining aqueous phase, consisting of distilled water, potassium sorbate and glycerin. The complete aqueous phase was then combined with the oil phase, prepared as a mixture of sunflower and plum oils. Emulsification was performed using a high-shear homogenizer operating at 10.000 rpm for 2 min. The resulting formulations were transferred into appropriate containers and allowed to equilibrate at room temperature before subsequent characterization. A summary of the tested microparticle types and incorporation quantity is reported in **Table 6.**

Table 6. Formulation ingredients (% w/w) for emulsion development.

Ingredients	F0	F1	F2	F3	F4
Aqueous phase					
Water	85.51	85.51	85.51	85.51	85.51
Glycerin	5	5	5	5	5
Potassium sorbate	0.2	0.2	0.2	0.2	0.2
Vivapur CS TEX Easy	5.50	2.75	2.75	2.75	1.5
Native tapioca starch	-	2.75	-	-	-
Tapioca with oleic acid	-	-	2.75	-	-
Tapioca with PMW	-	-	-	2.75	4
Oil phase					
Sunflower oil	2.53	2.53	2.53	2.53	2.53
Plum oil	1.25	1.26	1.26	1.26	1.26

Emulsion preparation with chitosan and CMC-based microparticles

For formulation F5, oleic acid–modified chitosan microparticles were incorporated in combination with Vivapur. The aqueous phase was prepared by dispersing oleic acid–modified chitosan in water containing potassium sorbate adjusted to pH 5.5 under mechanical stirring, while Vivapur was dispersed separately in water and allowed to hydrate for 15 min. The two aqueous dispersions were then combined, glycerin was added, and the mixture was homogenized until a uniform aqueous phase was obtained. The resulting aqueous phase was subsequently emulsified with the oil phase (sunflower and plum oils) using a high-shear homogenizer (Polytron PT 10–35 GT, Kinematica AG, Switzerland) at 10,000 rpm for approximately 2 min. For formulation F6, oleic acid–modified chitosan microparticles were used as the sole particulate stabilizer. The chitosan microparticles were dispersed in water containing potassium sorbate adjusted to pH 5.5 under mechanical stirring, followed by the addition of glycerin. The homogeneous aqueous phase was then combined with the oil phase and emulsified under the same high-shear conditions described above. For formulation F7, oleic acid–modified carboxymethylcellulose (CMC) microparticles were evaluated in combination with Vivapur. Oleic acid–modified CMC was dispersed in water under mechanical stirring, while Vivapur was hydrated separately in water for 15 min. The two dispersions were subsequently combined with glycerin to obtain an homogeneous aqueous phase, which was then emulsified with the oil phase using high-shear homogenization at 10,000 rpm for approximately 2 min. A summary of the tested microparticle types and incorporation quantity is reported in **Table 7**.

Table 7. Ingredients percentage composition (% w/w).

Ingredients	**F5**	**F6**	**F7**
Aqueous phase			
Water	85.51	85.51	85.51
Glycerin	5.00	5.00	5.00
Potassium sorbate	0.2	0.2	0.2
Vivapur CS TEX Easy	2.75		2.75
Chitosan with oleic acid	2.75	5.50	
CMC with oleic acid	-	-	2.75
Oil phase			
Sunflower oil	2.53	2.53	2.53
Plum oil	1.25	1.26	1.26

4.5. Characterization of the Emulsions

Each formulation was characterized in terms of organoleptic properties, pH, phase stability, accelerated stability, rheological behavior and droplet size distribution analysis.

Organoleptic properties

All formulations were characterized in terms of color and texture. The evaluations were performed under standardized conditions, including fixed lighting and location. Texture assessment was carried out by applying a small amount of formulation between the index and middle fingers in the palm hollow and gently spreading it by rubbing with two fingers, in order to qualitatively evaluate spreadability, consistency, and sensorial perception.

pH Determination

The pH of each sample was measured one day after the formulation was prepared at room temperature, using a digital pH meter (VWR avantor pH 1100 L Operating Manual), calibrated with standard buffers at pH 4.0, 7.0, and 9.0. The electrode probe was directly immersed in the emulsion.

Stability Analysis

The physical stability of the emulsions was assessed using a Turbiscan LAB analyzer (Formulaction, Toulouse, France). The instrument is equipped with a pulsed near-infrared light source (λ = 880 nm) and two synchronous detectors: transmission (T) and backscattering (BS). During the analysis, a scanning head moves along the height of the sample vial, recording BS and T signals across the emulsion column. After the emulsion formation, a fixed volume of sample was transferred into glass vials and analyzed under controlled conditions. The stability profile was monitored at different storage temperatures (4 °C, 25 °C, and 45 °C) and at defined time intervals (0 h, 1 h, 24 h, 48 h, and 7 days). The obtained backscattering (BS) curves, expressed as the percentage of reflected light as a function of sample height, provided information on destabilization phenomena such as droplet coalescence, creaming, or sedimentation.

Accelerated stability analysis

To predict physical instability phenomena, samples were subjected to strong mechanical stress conditions by using the centrifuge Eppendorf 5804 R. To perform the test, 10 g of each sample was placed in centrifugal tubes and centrifuged at 4000 rpm for 30 min at 25°C. This process causes stress in the sample, simulating an increase in gravity and causing more particle movement, which predicts future instabilities. Phase separation or the appearance of distinct layers was evaluated visually after centrifugation.

Rheological analysis

The rheological properties of the emulsions were characterized using a rheometer (Discovery HR10, TA instruments) with a parallel plate geometry (40.0 mm parallel plate, Peltier plate Sandblasted, Solvent Trap). The temperature was set at 25 °C. The shear rate explored ranged from 0.01 to 1000 s^{-1}. Viscosity values were recorded, and flow curves were analyzed to determine the shear-thinning behavior and structural stability of the emulsions. Amplitude sweep tests were performed, increasing the strain from 0.01% to 1000%, at a fixed oscillation frequency of 1 Hz, to identify the sample's linear viscoelastic region (LVER). All measurements were made in triplicate and data reported as mean ± SD.

Particle size analysis

The droplet size distribution of the emulsions was determined by laser diffraction using a laser diffraction particle size analyzer (Shimadzu, SALD-2300). Before analysis, each sample was diluted 1:10 by dispersing 1 g of emulsion in 9 mL of distilled water. The dispersion was stirred to obtain a homogeneous sample. The suspension was then introduced dropwise into the flow cell. Measurements were made using a liquid cell at room temperature using the Fraunhofer optical model, suitable for micron-sized samples with unknown refractive indices (set to 3.00 – 0.20i). Results are given as mean ± SD. The droplet size distribution was expressed as mean, median (D50), mode, standard deviation and percentile diameters (D10, D50, D90), reported in micrometers (µm).

Light microscopy

Emulsions were observed under a light microscope (Leica DMi8, inverted microscope, Germany) at ambient temperature. A drop of each sample was placed between a glass slide and a cover slip and observed immediately. Images were acquired at 10×, 20×, and 40× magnifications. For each sample, five micrographs were taken, and representative images were selected to illustrate the microstructure of the emulsions.

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Conclusions

This research study investigated the development of biodegradable systems for cosmetic applications by combining the design of polysaccharide-based microparticles with their functional integration into surfactant-free Pickering emulsions. The objective was to contribute to the replacement of persistent synthetic microplastics by sustainable alternatives while preserving formulation performance, stability, and sensory acceptability.

The first part of the thesis focused on the synthesis and physicochemical characterization of microparticles derived from starch, chitosan, and carboxymethylcellulose. Chemical modifications enabled tuning of surface polarity, dispersibility, and interfacial affinity, while systematic characterization established correlations between chemical structure, morphology, and particle size distribution. Among the materials studied, modified starch microparticles proved to be particularly promising, making them excellent candidates for replacing microplastics in cosmetic formulations.

The second part addressed the design of a sustainable Pickering emulsion platform based on a minimal ingredient approach and a multifunctional natural-based stabilizer. The application of a Design of Experiments (DoE) methodology proved effective in identifying optimal compositional range and capturing the interactive effects of formulation parameters on stability, microstructure, and rheological performance. The optimized formulation exhibited high physical stability, shear-thinning behavior, and viscoelastic characteristics compatible with consumer expectations and industrial processing requirements.

The incorporation of the synthesized microparticles into the optimized Pickering emulsion provided valuable insights into the practical limitations and opportunities associated with using biodegradable particles as functional stabilizers in complex cosmetic systems. Although the microparticles developed in this work did not yet enable the formation of fully particle-stabilized emulsions capable of replacing the Vivapur cellulose-based stabilizer, the study clearly demonstrated the strong influence of the particles on formulation behavior, stability, and sensory performance. Modified starch microparticles showed the highest degree of compatibility with the emulsion matrix, contributing to microstructural organization and rheological modulation while maintaining acceptable stability. In contrast, oleic acid–modified chitosan and carboxymethylcellulose microparticles led to reduced homogeneity and limited formulation consistency.

This study is a fundamental step towards rationally exploiting modified natural materials for sustainable cosmetic formulations rather than a definitive solution. The results generated in this thesis establish critical structure–property–performance relationships that can guide future developments, including optimization of surface modification degree, particle size distribution, wettability tuning, and dispersion strategies. With continued refinement, these biodegradable microparticles have the potential to evolve into effective functional stabilizers or hybrid systems, contributing to the progressive replacement of persistent synthetic microplastics and supporting the transition toward more environmentally responsible cosmetic technologies.

From an application-oriented perspective, the systems developed in this work show significant potential for industrial translation. The use of scalable and industry-compatible techniques, such as spray drying and antisolvent precipitation, combined with widely available and renewable raw materials, supports the feasibility of upscaling these formulations toward commercial production. In particular, the modular design of the developed microparticles allows for further optimization depending on the targeted application, enabling the tuning of surface properties, particle size, and functional performance. Beyond cosmetic applications, the physicochemical and interfacial properties of these biopolymer-based systems suggest potential applicability in adjacent sectors, including pharmaceutical, nutraceutical, and biomedical formulations, where biocompatibility, biodegradability, and controlled release are key requirements. In this context, Pickering emulsions stabilized by bio-based particles may represent a versatile platform for the encapsulation and delivery of active ingredients. Future research should focus on the optimization of formulation robustness under industrial processing conditions, long-term stability studies, sensory evaluation through consumer panels, and compatibility with active compounds. Overall, the results of this thesis provide a solid foundation for the development of next-generation sustainable cosmetic formulations and contribute to resolving the gap between laboratory-scale research and industrial implementation, supporting the transition toward greener and more circular product design.

Project 2:

IN VITRO CYTOTOXIC POTENTIAL OF MANGROVE *AVICENNIA MARINA*

This chapter describes a collaborative side project focused on the phytochemical characterization and biological evaluation of the mangrove plant *Avicennia marina*.

In parallel with the main doctoral project, I participated in a collaborative side study focused on the biological screening of plant extracts from *Avicennia marina*, a mangrove species native to the Arabian Gulf. Although this work is not directly connected to the main research topic of the present thesis, it is methodologically aligned with my doctoral training, as it involved *in vitro* biological evaluation, data analysis, and interpretation of bioactivity, which are highly relevant in the broader context of cosmetic science, where both functional performance and safety assessment of ingredients are essential. Moreover, plant-derived extracts such as Avicennia marina represent a valuable class of bioactive ingredients with potential applications in cosmetic formulations, for example, as antioxidant or anti-aging agents. From this perspective, the study complements the main focus of the thesis by addressing another key aspect of formulation development, namely the identification and evaluation of functional active compounds.

My contribution was specifically limited to the *in vitro* cytotoxicity evaluation of plant extracts, within a multidisciplinary project that combined phytochemical profiling, antioxidant assays, and computational predictions.

This chapter has been adapted and modified from the following published work:

Cerri, F., Giustra, M., Anadol, Y., Tomaino, G., Galli, P., Labra, M., Campone, L., Colombo,M. (2022). Natural products from mangroves: an overview of the anticancer potential of
Avicennia marina. *Pharmaceutics*, 14(12), 2793.

1. Background

Avicennia marina, commonly known as the grey mangrove, is a mangrove belonging to the Acanthaceae family [1].It is widely distributed across tropical and subtropical regions, including Africa; South, Southeast, and Southwest Asia; the Malay Archipelago; the North Island of New Zealand; Australia; the Southwest Pacific; and the Maldives [1], [2], [3]. It is a plant highly resistant to extreme environmental conditions, such as high salinity, high temperature, strong winds, and anaerobic soil. These extraordinary resistance capacities prompted researchers to investigate the potential of this plant for pharmaceutical applications and the development of new drugs.

Extensive studies on *A. marina* have revealed a complex chemical profile, encompassing diverse classes of bioactive compounds such as flavonoids, iridoid glycosides, terpenoids, alkaloids, and steroids, along with a variety of other secondary metabolites [3], [4], [5], [6].

A. marina extracts and isolated compounds have demonstrated a wide range of diverse biological activities, including antimalarial, antibacterial, analgesic, antioxidant, antifouling, antidiabetic, anti-inflammatory, anti-rheumatoid arthritis, and anticancer effects [7]. Among these, *A. marina* extracts have demonstrated promising in vitro anticancer activity [3]. However, studies on this species remain limited, particularly in the Arabian Gulf, where it dominates coastal mangrove forests in the UAE, Saudi Arabia, Bahrain, Qatar, and Iran [8]. Ethnobotanical data in this region are scarce, with reported traditional uses ranging from treatments of ulcers and rheumatism in Iran to applications as aphrodisiac, antifertility agent, and remedies for scabies and toothache in the UAE [9]. The extreme environmental conditions of the Arabian Gulf, high temperature, hypersalinity, and elevated turbidity, may induce unique metabolic adaptations in *A. marina*, promoting the accumulation of secondary metabolites such as flavonoids, iridoid glycosides, and phenylethanoid glycosides, which contribute to stress tolerance and possess pharmacologically relevant antioxidant and anticancer activities [10]. In this context, the present study aimed to evaluate the in vitro cytotoxic potential of mangrove *Avicennia marina* extracts.

2. Results

2.1 In vitro cytotoxic activity

The cytotoxic effects of *A. marina* extracts (leaf, cotyledon, pericarp, propagule, and root) were evaluated against a panel of human cancer cell lines using the MTT assay. Four concentrations (20, 60, 180, and 540 µg/mL) were tested on two colorectal cancer cell lines (SW480 and E705) (**Figure 2.1.1)** and three additional cancer cell lines: MDA-MB-231 (triple-negative breast cancer), U-87 (glioblastoma), and HeLa (cervical cancer) (**Figure 2.1.2**). Furthermore, two non-cancerous cell lines, MRC-5 (normal human fibroblasts) and CCD 841 (healthy human mucosa), served as controls to assess extract selectivity (**Figure 2.1.3).**

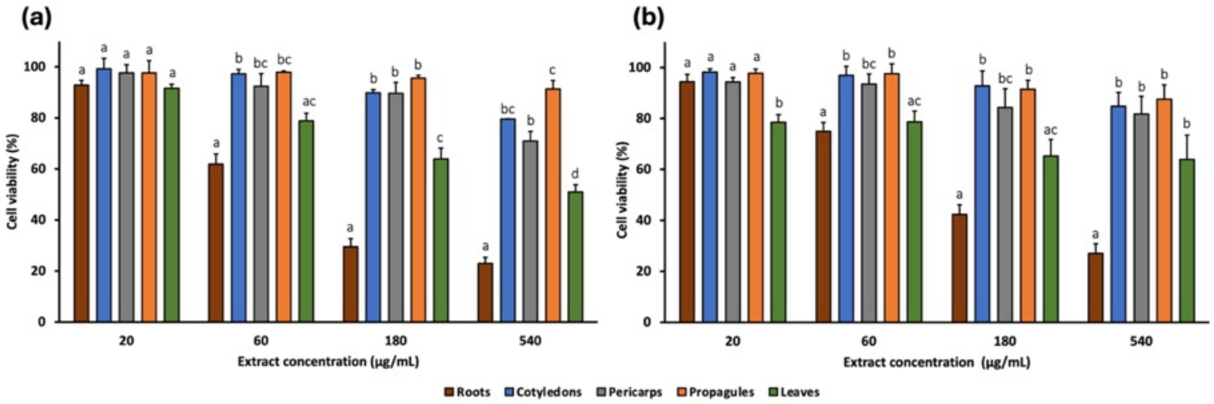

Figure 2.1.1. Cell viability (%) of the human colorectal cancer cell lines SW480 (a) and E705 (b) following 48 h treatment with *Avicennia marina* extracts at concentrations ranging from 20 to 540 µg/mL. Data are expressed as mean ± standard error of the mean (SEM) from three independent experiments (n = 3). Different lowercase letters denote statistically significant differences among extracts ($p < 0.05$) and were assigned separately for each tested concentration.

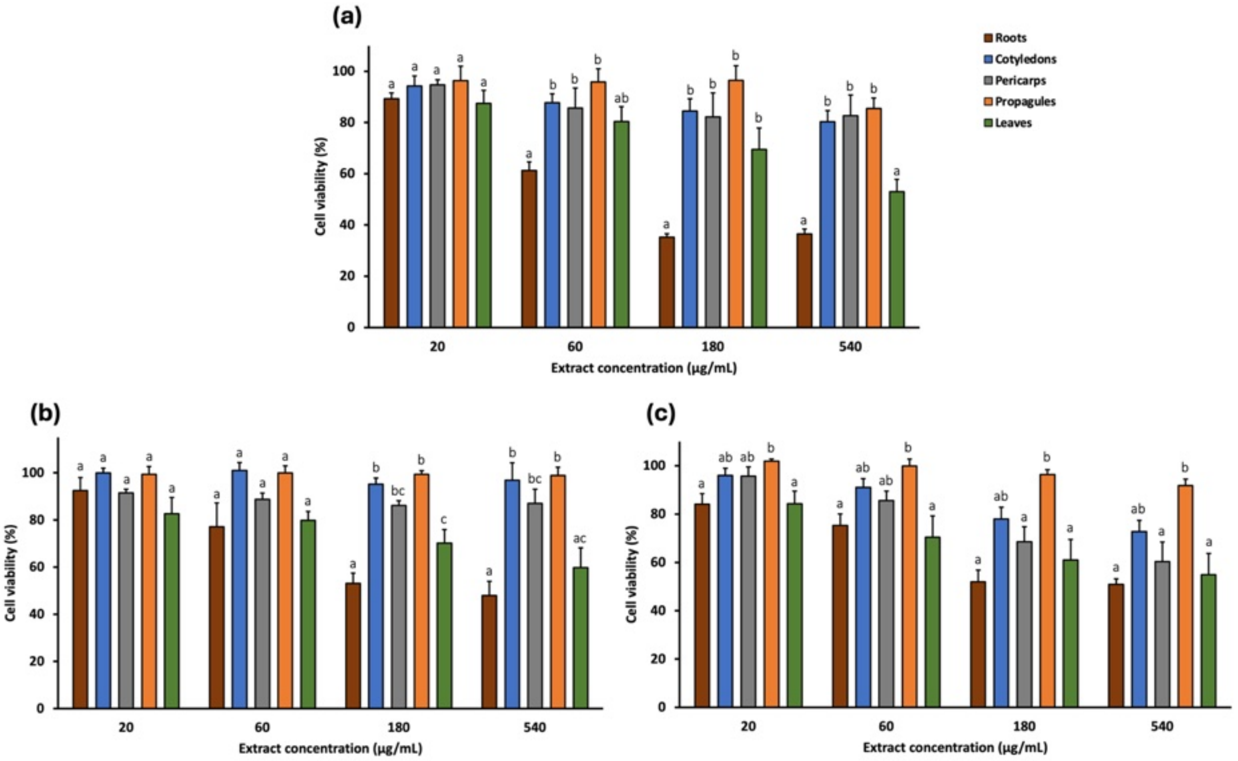

Figure 2.1.2 Cell viability (%) of the human cancer cell lines MDA-MB-231 (a), U-87 (b), and HeLa (c) after 48 h exposure to *Avicennia marina* extracts at concentrations ranging from 20 to 540 µg/mL. Data are presented as mean ± standard error of the mean (SEM) from three independent experiments (n = 3). Different lowercase letters indicate statistically significant differences among extracts ($p < 0.05$) and were assigned separately for each concentration.

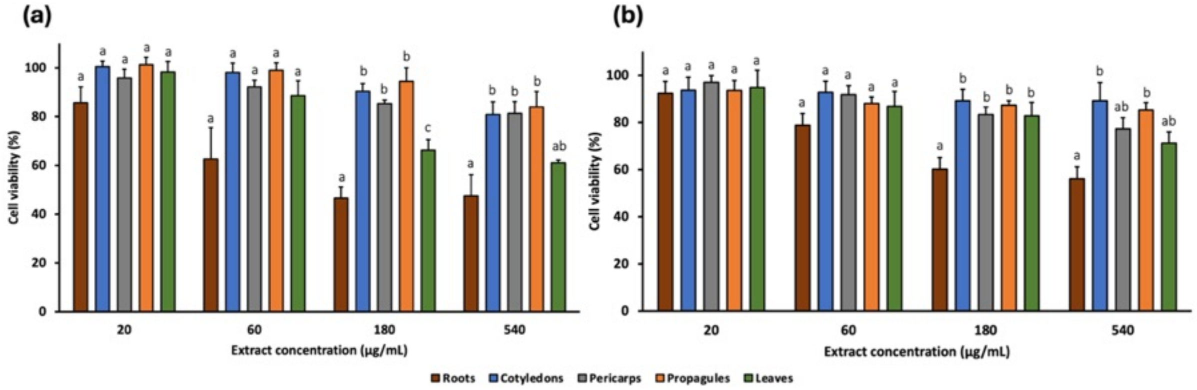

Figure 2.1.3 Cell viability (%) of the healthy human cell lines CCD 841 (a) and MRC-5 (b) following 48 h treatment with *A. marina* extracts at concentrations ranging from 20 to 540 µg/mL. Data are presented as mean ± standard error of the mean (SEM) from three independent experiments (n = 3). Different lowercase letters denote statistically significant differences among extracts ($p < 0.05$) and were assigned independently for each concentration.

Among the tested extracts, those obtained from cotyledons, pericarps, and propagules exhibited overall low cytotoxic activity, with cell viability remaining above 70% even at the highest tested concentration (540 µg/mL) in all cell lines. An exception was observed for the pericarp extract, which reduced HeLa cell viability to 60.30% at 540 µg/mL. The leaf extract showed limited cytotoxic effects at lower concentrations. At 60 µg/mL, cell viability was approximately 80% in SW480, E705, and MDA-MB-231 cells, and 70.44% in HeLa cells. In non-cancerous cell lines, viability was higher, reaching 88.59% in CCD 841 and 86.86% in MRC-5 cells. At the highest concentration tested (540 µg/mL), the leaf extract induced a more pronounced reduction in viability (approximately 50–60%) in most cell lines, including SW480 (50.98%), E705 (63.91%), MDA-MB-231 (53.00%), U-87 (59.77%), HeLa (54.96%), and CCD 841 (61.01%), whereas MRC-5 cells were less affected (71.26%).

In contrast, the root extract exhibited the strongest cytotoxic activity among all samples. At 180 µg/mL, cell viability decreased to 29.47% in SW480, 42.40% in E705, 35.26% in MDA-MB-231, 53.06% in U-87, 52.00% in HeLa, 46.60% in CCD 841, and 60.15% in MRC-5 cells. These reductions were statistically significant compared to those induced by the other extracts, except for E705 (where differences from the leaf extract were not significant) and HeLa cells. At 540 µg/mL, cytotoxic effects remained comparable across most cell lines, with a further decrease in viability observed in SW480 and E705 cells (22.93% and 27.03%, respectively).

Overall, these results underscore the pronounced cytotoxic potential of the root extract, particularly at higher concentrations, against SW480, E705, and MDA-MB-231 cancer cell lines (**Figure 2.1.4**). Notably, at 180 µg/mL, the root extract exhibited significantly greater cytotoxic effects on these cancer cell lines than on non-cancerous MRC-5 cells, while no significant difference was detected when compared with the healthy mucosa cell line CCD 841. Moreover, at 540 µg/mL, SW480 cell viability was significantly lower than that of CCD 841, indicating a degree of selectivity toward cancer cells.

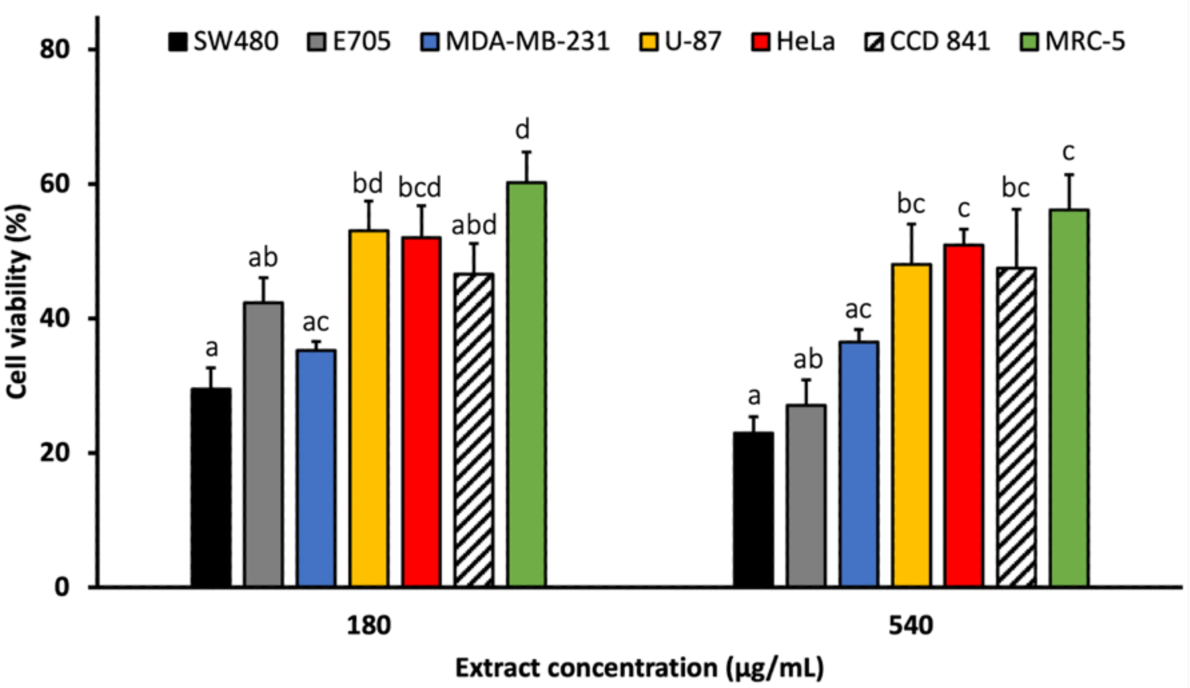

Figure 2.1.4 Cell viability (%) of cancer cell lines after 48 h exposure to *A. marina* root extract at 180 and 540 µg/mL. Results are expressed as mean ± standard error of the mean (SEM) from three independent experiments (n = 3). Different lowercase letters indicate statistically significant differences between treatments ($p < 0.05$) and were assigned independently for each concentration.

Given this pronounced response, the analysis focused on dose–response effects in SW480, E705, and MDA-MB-231. Each cell line was treated with ten increasing concentrations of root extract (2 to 540 µg/mL (**Figure 2.1.5**), and the IC50 values were 81.98 µg/mL for SW480, 108.10 µg/mL for E705, and 57.93 µg/mL for MDA-MB-231.

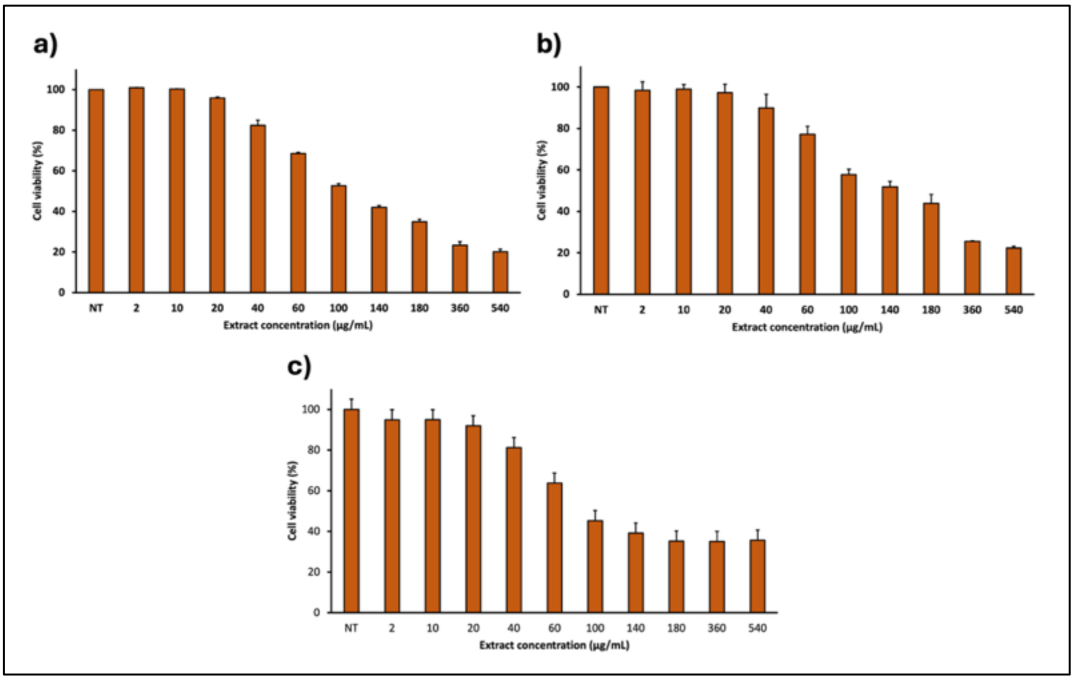

Figure 2.1.5 Cell viability of SW480 (a), E705 (b), and MDA-MB-231 (c) human cancer cell lines treated with root extract (2–540 µg/mL) for 48 h. Average values ± SEM are shown.

3. Discussion

Regarding the cytotoxic activity, cotyledon, pericarp, and propagule extracts exhibited negligible effects on all tested cancer cell lines, even at the highest concentrations. In contrast, leaf and root extracts showed dose-dependent cytotoxicity. The leaf extract was weakly active at low concentrations but reduced cell viability to approximately 50–60% at 540 µg/mL in several cancer cell lines, in agreement with previous findings reporting limited toxicity at low doses and moderate effects at higher concentrations. Among all samples, the root extract demonstrated the most pronounced cytotoxic activity, particularly against SW480, E705, and MDA-MB-231 cell lines. At 540 µg/mL, cell viability dropped below 40%, while lower toxicity was observed in non-cancerous cell lines (MRC-5 and CCD 841). The IC_{50} values for SW480, E705, and MDA-MB-231 were 81.98, 108.10, and 57.93 µg/mL, respectively, corresponding to moderate cytotoxic activity according to the National Cancer Institute (NCI) and Geran protocol which classified cytotoxicity as high when IC50 values are ≤ 20 µg/mL, moderate between 21 and 200 µg/mL, weak between 201 and 500 µg/mL, and absent above 500 µg/mL [10]. Although no direct comparison with a reference anticancer drug was performed, these results indicate the presence of biologically active constituents in the crude extracts. Notably, the observed cytotoxicity may be attributed to triterpene saponins, which were exclusively detected in the root extract. These compounds are increasingly recognized for their anticancer potential combined with relatively low toxicity. Given the superior activity of the root extract, future studies will focus on bioactivity-guided fractionation, particularly targeting saponin-rich fractions. These fractions will be evaluated alongside standard anticancer drugs (e.g., doxorubicin) and subjected to mechanistic investigations, including apoptosis induction, cell cycle modulation, and pathway analysis. Such integrated chemical and biological approaches are expected to clarify structure–activity relationships and support the identification of promising lead compounds for subsequent in vivo evaluation and anticancer drug development.

4. Materials and Methods

4.1. Plant material

Samples were collected from different parts of *A. marina*, including leaves, roots, propagules, and cotyledons. The cotyledons were obtained from seedlings at an early growth stage, when the propagules had already opened and developed roots. All samples were harvested in September 2022 from multiple individual plants within the mangrove forest of Ajman Emirate, UAE. Although no herbarium voucher specimen was deposited, the plant material was identified based on morphological characteristics following established taxonomic keys. This identification is supported by the fact that *A. marina* is the only mangrove species forming the evergreen coastal forests of the UAE, and its presence in the region has been validated by previous molecular analyses.

Propagules were separated into the pericarp, representing the external protective tissue and the internal tissues. Thus, in the manuscript, the term pericarp (and pericarp extract) refers exclusively to the external part, while the generic term propagule (and propagule extract) refers to the internal tissues. Each type of plant material (e.g., all collected leaves, roots, pericarps, propagules, and cotyledons) was pooled by type and immediately freeze-dried after collection. The dried samples were homogenized using a Grindomix GM 200 knife mill (Retsch, Haan, Germany) and then sieved through a test sieve (Retsch AS 200, Haan, Germany) with a mesh size range of 300–600 µm to obtain powders with uniform particle size distribution.

4.2. Sample preparation and extraction

Root, leaf, cotyledon, pericarp, and propagule samples of *A. marina* underwent exhaustive ultrasound-assisted extraction using a thermostatically controlled ultrasonic bath (Sonorex TK 52; Bandelin electronic, Berlin, Germany). Each sample was extracted under controlled conditions (25 °C, 15 min) with 50% aqueous ethanol (*v*/*v*) at a solid-to-solvent ratio of 1:10 (*w*/*v*), which is commonly applied in metabolite profiling studies; specifically, 1 g of powdered sample was mixed with 10 mL of solvent in a 50-mL polypropylene tube. A 50% aqueous ethanol solution was selected as the extraction solvent due to its effectiveness as a green, low-toxicity system, making it well-suited for bioactivity screening. Ethanol–water mixtures offer a balanced polarity and are widely recognized for their ability to efficiently extract a broad range of bioactive compounds, particularly polyphenolic metabolites, which are well known for their antioxidant properties. Moreover, this solvent was selected to ensure low toxicity in downstream biological assays, in case traces of solvent remain after evaporation, and because non-polar solvents or higher ethanol concentrations could reduce solubility in aqueous assay media, potentially compromising the suitability of the extracts for biological testing.

To ensure complete extraction, the process was repeated three times with fresh solvent. Following each extraction, the mixtures were centrifuged (13,000× *g*, 10 min), and the supernatants underwent filtration through Whatman No. 1 filter paper. The combined extracts were concentrated under pressure at 40 °C using a rotary evaporator to remove ethanol and subsequently lyophilized (Alpha 1-2 LD freeze dryer, Christ, Germany) to obtain dry residues for further analysis.

The extraction yields of *A. marina* were determined by calculating the ratio of the weight of dried extract obtained to the initial weight of dried plant material powder and expressed as a percentage. The yields were 34.23% for roots, 65.02% for cotyledons, 61.76% for pericarps, 38.55% for propagules, and 33.72% for leaves.

4.3. Cytotoxic evaluation

Cell lines and culture conditions

Normal human fibroblasts (MRC-5), human glioblastoma (U-87), human triple-negative breast cancer (MDA-MB-231), human colorectal cancer (SW480), and human healthy mucosa (CCD841) cell lines were purchased from the American Type Culture Collection (Manassas, VA, USA). The human cervical cancer cell line (HeLa) was acquired from System Biosciences, and the human colon cancer cell line (E705) was provided by the Fondazione IRCCS Istituto Nazionale dei Tumori (Milan, Italy). The E705 cell line represents epithelial tissue cells of colorectal adenocarcinoma derived from a patient at the National Cancer Institute in Milan. MRC-5, U-87, and HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) high glucose medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin/streptomycin (P/S), and 2 mM L-glutamine. MDA-MB-231 cells were maintained in Minimum Essential Medium (MEM) with Earl's Salts supplemented with 10% heat-inactivated FBS, 1% P/S, 2 mM L-glutamine, and 0.1 mM MEM Non-Essential Amino Acids (MEM NEAA). E705 and SW480 cell lines were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamine. The CCD 841 cell line was grown in EMEM medium supplemented with 10% heat-inactivated FBS, 1% P/S, 2 mM L-glutamine, and 0.1 mM non-essential amino acids. All cell lines were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air. Cell culture media and reagents were purchased from EuroClone (Pero, Italy).

Viability assay

The cytotoxicity of *A. marina* extracts was evaluated using the MTT assay (CellTiter96®Non-Radioactive Cell Proliferation Assay, Promega, Madison, WI, USA) following the 64 manufacturer’s protocol. The extract powders were solubilized in Milli-Q water, and four extract concentrations (20, 60, 180, and 540 µg/mL) were tested on all cell lines. Additionally, for the root extract, an extended dose-response analysis using ten concentrations (2, 10, 20, 40, 60, 100, 140, 180, 360, and 540 µg/mL) was performed on selected cell lines to enable IC_{50} determination. IC_{50} values were calculated only for extract–cell line combinations tested with this ten-point dilution series.

No positive control drugs were included in this preliminary screening, as the primary objective was to assess and compare the relative cytotoxicity of different *A. marina* extracts. Comparative analysis with standard anticancer agents will be incorporated in subsequent studies on purified fractions or isolated compounds.

Briefly, HeLa, MRC-5, U-87, and MDA-MB-231 cells were seeded into 96-well plates (from Euroclone, Pero, Italy) at a density of $5 \times 10_3$ cells/well in 100 µL of growth medium, while E-705 and SW-480 cells were seeded at a density of $8 \times 10_3$ cells/well. After 24 h of incubation at 37 °C in 5% CO_2, the medium was replaced, and cells were treated with various concentrations of *A. marina* extracts. Following 48 h of treatment, the medium was replaced, and 15 µL of MTT solution was added to each well. After 3 h of incubation at 37 °C, formazan crystals were solubilized using 100 µL of stop solution and incubated under stirring for 1 h. Reduced MTT was quantified using a UV–vis plate reader (EnSight Multimode Microplate Reader, PerkinElmer, Waltham, MA, USA) at 570 nm with a reference wavelength of 630 nm. Cell viability was expressed as a percentage relative to untreated cells (negative control), and medium with MilliQ water at equivalent concentrations (10% *v*/*v*) was used as a blank. Dose-response curves and the IC_{50} values, representing the extract concentration required to inhibit 50% of cell viability relative to untreated control cells, were generated using GraphPad Prism v10.5.0 software.

4.4. Statistical analysis

Statistical analyses were conducted on data generated from three replicates. Cytotoxic activity results are presented as mean ± standard error of the mean (SEM).

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Conclusions

This study identifies *Avicennia marina* as a promising source of bioactive secondary metabolites with potential therapeutic relevance. Among the investigated extracts, the root extract showed the most pronounced cytotoxic activity, particularly against the triple-negative breast cancer cell line MDA-MB-231 and the colorectal cancer cell lines SW480 and E705, with IC_{50} values of 58.46, 81.98, and 108.10 µg/mL, respectively. To translate these findings toward pharmacological relevance, future studies should prioritize bioactivity-guided fractionation, structural elucidation, and targeted quantification of key metabolites, coupled with mechanistic in vitro investigations. Such approaches will be essential to clarify structure–activity relationships and may ultimately support the identification of novel therapeutic leads from this stress-adapted mangrove species.

List of publications

1. Francesca Spena†, Beatrice De Santes, Lucia Morelli, Richard Daniellou, Delphine Huc-Mathis, Lucia Salvioni, Paolo Galli, Davide Prosperi, Marco Giustra, Miriam Colombo (2026). From biopolymers to Pickering emulsions: a green chemistry strategy to replace microplastics in next-generation cosmetics, RSC Advances,16 (22), 2026, 20399-20410.
   https://doi.org/10.1039/d6ra01252c
   † First author

2. Cerri, F.,* De Santes, B.,* Spena, F.,* Salvioni, L., Forcella, M., Fusi, P., ... & Campone, L. (2025). Phytochemical profiling, antioxidant activity, and in vitro cytotoxic potential of mangrove Avicennia marina. Pharmaceuticals, 18(9), 1308.
   *Equal contribution

3. Giustra, M., Sinesi, G., Spena, F., De Santes, B., Morelli, L., Barbieri, L., ... & Colombo, M. (2024). Microplastics in cosmetics: open questions and sustainable opportunities. ChemSusChem, 17(22), e202401065.

4. Morelli, L., Ochoa, E., Salvioni, L., Giustra, M. D., De Santes, B., Spena, F., ... & Colombo, M. (2024). Microfluidic nanoparticle synthesis for oral solid dosage forms: A step toward clinical transition processes. International Journal of Pharmaceutics, 652, 123850.

5. Ochoa, E., Morelli, L., Salvioni, L., Giustra, M., De Santes, B., Spena, F., ... & Colombo, M. (2024). Co-processed materials testing as excipients to produce Orally Disintegrating Tablets (ODT) using binder jet 3D-printing technology. European Journal of Pharmaceutics and Biopharmaceutics, 194, 85-94.

6. Beatrice De Santes, Lucia Morelli, Francesca Spena, Stefania Pagliari, Delphine Huc-Mathis, Richard Daniellou, Marco Giustra, Luca Campone, Federico Cerri, Paolo Galli, Davide Prosperi, Lucia Salvioni*, Miriam Colombo
   Prosopis cineraria (Ghaf) Extract as a Natural Ingredient for Anti-Aging Dermocosmetic Applications (submitted).

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Article

Phytochemical Profiling, Antioxidant Activity, and In Vitro Cytotoxic Potential of Mangrove *Avicennia marina*

Federico Cerri [1,2,†], Beatrice De Santes [1,3,†], Francesca Spena [1,3,†], Lucia Salvioni [3,4], Matilde Forcella [3], Paola Fusi [3], Stefania Pagliari [3,*], Henrik Stahl [5], Paolo Galli [1,2], Miriam Colombo [3,4], Marco Giustra [3,4,*] and Luca Campone [3]

[1] Department of Earth and Environmental Sciences DISAT, University of Milano-Bicocca, Piazza della Scienza 1, 20126 Milan, Italy; federico.cerri@unimib.it (F.C.); b.desantes@campus.unimib.it (B.D.S.); f.spena2@campus.unimib.it (F.S.); paolo.galli@unimib.it (P.G.)
[2] MaRHE Centre (Marine Research and Higher Education Center), Magoodhoo Island, Faafu Atoll 12030, Maldives
[3] Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milan, Italy; lucia.salvioni@unimib.it (L.S.); matilde.forcella@unimib.it (M.F.); paola.fusi@unimib.it (P.F.); miriam.colombo@unimib.it (M.C.); luca.campone@unimib.it (L.C.)
[4] Nanomedicine Center NANOMIB, University of Milano-Bicocca, 20854 Vedano al Lambro, Italy
[5] College of Marine Science and Aquatic Biology, University of Khorfakkan, Sharjah 18119, United Arab Emirates; henrik.stahl@ukf.ac.ae
[*] Correspondence: stefania.pagliari@unimib.it (S.P.); marco.giustra@unimib.it (M.G.)
[†] These authors contribute equally to this work.

Abstract

Background: *Avicennia marina* (Forsk.) Vierh., a widely distributed mangrove species, is known for its diverse secondary metabolites with potential pharmacological applications. Despite its dominance in the Arabian Gulf, where *A. marina* may have adapted to extreme environmental conditions with a distinct set of bioactive molecules, research in this region remains limited. **Methods:** This study investigates the phytochemical composition, antioxidant activity, and in vitro cytotoxicity of extracts from different plant parts, including roots, leaves, propagules, pericarps, and cotyledons, collected in the United Arab Emirates (UAE). Extracts were analyzed using ultra-pressure liquid chromatography coupled with high-resolution mass spectrometry (UPLC-HRMS). Antioxidant activity was assessed using DPPH and ABTS assays, while cytotoxicity was evaluated against human cancer and normal cell lines. **Results:** Analysis revealed 49 compounds, including iridoid glycosides, hydroxycinnamic acids, phenylethanoid glycosides, flavonoid glycosides, and triterpene saponins, several reported for the first time in *A. marina* and mangroves. The pericarp and root extracts exhibited the highest scavenging activity (DPPH: 187.14 ± 2.87 and 128.25 ± 1.12; ABTS: 217.16 ± 2.67 and 147.21 ± 2.42 µmol TE/g, respectively), correlating with phenylethanoid content. The root extract also displayed the highest cytotoxicity, with IC_{50} values of 58.46, 81.98, and 108.10 µg/mL against MDA-MB-231, SW480, and E705, respectively. In silico analysis identified triterpene saponins as potential contributors. **Conclusions:** These findings highlight the root extract of *A. marina* as a promising source of bioactive compounds with potential antioxidant and anticancer applications, supporting further exploration for novel therapeutic candidates.

Keywords: mangroves; *Avicennia marina*; natural products; bioactive compounds; phytochemical analysis; UPLC-HRMS; antioxidant activity; cytotoxicity; triterpene saponins

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1. Introduction

Avicennia marina (Forsk.) Vierh, commonly known as the grey mangrove, is a true mangrove species [1] belonging to the Acanthaceae family. It is widely distributed across tropical and subtropical regions, including Africa; South, Southeast, and Southwest Asia; the Malay Archipelago; the North Island of New Zealand; Australia; the Southwest Pacific; and the Maldives [1–3].

The chemical profile of *A. marina* has been extensively studied, leading to the identification of diverse classes of bioactive molecules, including flavonoids, iridoid glycosides, terpenoids, alkaloids, and steroids, as well as a wide range of other metabolites [3–8].

Traditionally, *A. marina* has been utilized in folk medicine across different countries to treat several ailments and diseases [9,10]. Furthermore, its extracts and isolated compounds have demonstrated a wide range of biological activities, including antiviral, antimicrobial, anthelmintic and antimalarial, analgesic, antioxidant, antifouling, anticancer, antidiabetic, and anti-inflammatory [3,11]. In particular, extracts of this species have demonstrated promising in vitro anticancer activity [3]. However, research in this field remains relatively limited, especially in the context of the Arabian Gulf, where *A. marina* is the dominant mangrove species along the coasts of the United Arab Emirates (UAE), Saudi Arabia, Bahrain, Qatar, and Iran [12]. Ethnobotanical records for *A. marina* in the Arabian Gulf are scarce but include traditional uses in Iran for treatments of ulcers, rheumatism, and burns [9], and in the UAE for its use as an aphrodisiac, antifertility agent, and treatment for scabies and toothache [13]. *A. marina* remains largely unexplored here in terms of bioprospecting, with most studies focusing instead on its distribution, ecological significance, ecosystem services, and management and conservation [14,15]. Phytochemical investigations have only examined populations from China, India, Pakistan, Egypt, other Indo-Pacific locations, and the Red Sea coasts of Saudi Arabia [10,11,16–18].

Previous phytochemical studies on *A. marina* have generally examined only a few plant parts, typically aerial parts and primarily leaves, lacking a comprehensive analysis of plant-part-specific secondary metabolites. Biological activity also presents limitations, often focusing on few plant parts and, in the case of cytotoxicity, testing only a small number of cancer cell lines [9–11]. A major gap in existing research of *A. marina* is the absence of integrated studies combining detailed phytochemical profiling with antioxidant and cytotoxic assays across multiple parts of the plant, which is essential for linking bioactivities to tissue-specific metabolites. Such combined strategies are well established in plant research [19,20] and have been applied to mangroves [21] as they facilitate the prioritization of promising extracts and compounds, thereby enhancing efficiency in the early discovery of bioactive molecules. Moreover, the majority of chemical studies have relied on GC-MS, which biases detection towards volatile constituents [3,11,16]. Although GC-MS can also characterize phenolic compounds, abundant in mangroves [22], it requires a derivatization step that is both complex and time-consuming [23]. In contrast, ultra-high performance liquid chromatography coupled with high-resolution mass spectrometry (UPLC-HRMS) has emerged as a powerful platform for untargeted metabolomic profiling of complex plant matrices, offering superior sensitivity, selectivity, and mass accuracy, and enabling comprehensive detection and identification of secondary metabolites, especially phenolics [24–27].

In addition to these methodological gaps, the limited geographical scope of previous studies represents a crucial limitation in fully understanding the phytochemical diversity and bioactivity of *A. marina*. The Arabian Gulf is characterized by extreme environmental conditions, including elevated seawater temperatures, hypersalinity, and high turbidity, driven by its arid climate and shallow basin [28], and summer air temperatures can reach 50 °C [29], further stressing local ecosystems. Since the chemical composition of plants is influenced by geographical, environmental, and climate factors [30–32], plants exposed to such stresses often respond by increasing the accumulation of secondary metabolites, such as flavonoids, iridoid glycosides, and phenylethanoid glycosides, which enhance their tolerance to adverse conditions and also possess bioactivities of pharmacological interest, including antioxidant and anticancer [33–39].

It is plausible that *A. marina* in the Arabian Gulf may have adapted to extreme conditions through metabolic changes and the induction of antioxidant defense systems [40], potentially resulting in a distinct set of secondary metabolites with unique biological activities. Consequently, the objective of this study is to conduct a comprehensive investigation of *A. marina* grown in the UAE by characterizing the secondary metabolite composition of multiple parts of the plant, including roots, leaves, propagules (pericarps and internal tissues), and cotyledons, and evaluating their potential health benefits through antioxidant and cytotoxic activity assays in vitro.

Unlike previous studies, this work employs UPLC-HRMS to present a detailed phytochemical profile of each tissue type, allowing for the identification of specific compounds of *A. marina* and their localization within the plant. Furthermore, while the antioxidant and anticancer potentials of *A. marina* extracts have been previously reported [3,11], this study provides a comprehensive evaluation of antioxidant and cytotoxic activities of all plant parts, along with expanded cytotoxic screening in multiple cancer cell lines. In addition, in silico predictions of biological activities were applied to compounds identified in the extracts. This multi-level approach addresses existing regional and methodological gaps and lays the groundwork for the discovery of bioactive compounds from plants adapted to extreme environmental conditions.

2. Results

2.1. Characterization of Avicennia marina Extracts

Roots, leaves, cotyledons, pericarps, and propagules of *A. marina* displayed distinct metabolite profiles. The chromatographic profiles of the extracts are provided in Supplementary Material (Figures S1–S5), and the list of tentatively identified compounds is shown in Table 1, along with their corresponding identification level (IL), which reflects the confidence of compound annotation based on MSI guidelines (see Section 4.4). For compounds assigned to IL2, the identification relied on comparisons of MS/MS fragmentation data with published spectra from the literature or spectral databases. The specific references used to support each IL2 assignment are included directly in the table.

Table 1. UHPLC-ESI/HRMS data of compounds detected in *Avicennia marina* extracts. The main fragment ion for each compound is indicated in bold.

No.	RT (min)	$[M-H]^-$	Formula	Δ ppm	MS/MS	Name	Class	Part	IL	Ref.
1	0.58	701.1893 $[M+Cl]^-$	$C_{24}H_{42}O_{21}$	−2.1677	665.2134, 485.1499, 443.1393, **383.1182**, 341.1075, 179.0549	Stachyose	Tetrasaccharides	Cotyledons/pericarps/propagules/roots	IL2	[41]
2	0.99	191.0188	$C_6H_8O_7$	6.3850	111.0073	Citric acid	Tricarboxylic acids	Cotyledons/pericarps	IL2	[42]
3	3.87	373.1139	$C_{16}H_{22}O_{10}$	0.3221	211.0605, 167.0700, 149.0597, **123.0440**, 105.0333	Geniposidic acid	Iridoid glycosides	Leaves/cotyledons/pericarps/propagules/roots	IL2	[43]
4	4.03	353.0875	$C_{16}H_{18}O_9$	0.8633	**191.0551**, 179.0339, 161.0233, 135.0439	Caffeoylquinic acid isomer	Hydroxycinnamic acids and derivatives	Roots	IL2	[44]
5	4.05	375.1291	$C_{16}H_{24}O_{10}$	1.5167	213.0756, 169.0857, **151.0753**, 133.0644, 125.0595, 107.0490	Mussaenosidic acid	Iridoid glycosides	Leaves/cotyledons/pericarps/propagules	IL2	[45]
6	4.33	375.1285	$C_{16}H_{24}O_{10}$	3.1119	213.0747, 169.0854, **151.0748**, 133.0644, 125.0591, 113.0230, 107.0484	(Epi)loganic acid	Iridoid glycosides	Leaves	IL2	[45]
7	4.53	487.1451	$C_{21}H_{28}O_{13}$	1.2588	**179.0334**, 161.0228, 135.0435	Cistanoside F	Phenylethanoid glycosides	Pericarps	IL2	[46]
8	4.80	327.0715	$C_{14}H_{16}O_9$	1.9983	179.0335, 165.0389, 147.0283, **135.0434**, 105.0178	Unidentified	-	Leaves		
9	5.09	353.0871	$C_{16}H_{18}O_9$	−3.0904	**191.0550**, 179.0337, 173.0442, 161.0232, 135.0439,	Caffeoylquinic acid isomer	Hydroxycinnamic acids and derivatives	Leaves/cotyledons/pericarps/propagules/roots	IL2	[44]
10	5.47	371.0982	$C_{15}H_{16}O_{11}$	−8.7858	**209.0635**, 179.0337, 161.0228, 135.0435, 129.0178	Caffeoyl hexaric acid	Hydroxycinnamic acids and derivatives	Leaves	IL2	[47]
11	5.60	443.0655	$C_{18}H_{20}O_{11}S$	−0.3243	275.0218, **167.0338**, **152.0105**, 123.0440, 108.0204	Unidentified	-	Roots		
12	5.65	415.1603	$C_{19}H_{28}O_{10}$	1.6114	235.0963, **191.1062**, 173.0958, 149.0953, 137.0590, 101.0226	Icariside D1	Flavonoid glycosides	Leaves	IL2	[48]
13	5.93	639.1964	$C_{29}H_{36}O_{16}$	−5.2193	621.1807, 529.1554, 459.1488, 251.0549, 179.0337, **161.0232**, 151.0387	Suspensaside isomer	Phenylethanoid glycosides	Pericarps/roots	IL2	[49,50]

Table 1. *Cont.*

No.	RT (min)	$[M-H]^-$	Formula	Δ ppm	MS/MS	Name	Class	Part	IL	Ref.
14	5.95	639.1964	$C_{29}H_{36}O_{16}$	−5.2193	621.1807, 529.1554, 459.1488, 251.0549, 179.0337, **161.0232**, 151.0387	Suspensaside isomer	Phenylethanoid glycosides	Pericarps/roots	IL2	[49,50]
15	6.14	537.1628	$C_{25}H_{30}O_{13}$	−2.6672	493.1708, 375.1275, 323.0758, 213.0752, 179.0334, 169.0854, **161.0230**, 151.0750, 135.0435, 125.0593, 107.0486	Grandifloroside	Hydroxycinnamic acid and derivatives	Leaves	IL2	[51]
16	6.33	619.1644	$C_{29}H_{32}O_{15}$	3.9407	383.0758, **311.0549**, 267.0646	Unidentified	-	Pericarps/roots		
17	6.41	639.1929	$C_{29}H_{36}O_{16}$	0.2477	**621.1817**, 529.1554, 459.1493, 251.0549 **179.0338**, 161.0236, 151.0385	Suspensaside isomer	Phenylethanoid glycosides	Roots	IL2	[49,50]
18	6.65	521.1658	$C_{25}H_{30}O_{12}$	1.2446	357.1176, 169.0854, 163.0385, 151.0749, 145.0280, 125.0591, 119.0486, 117.0329, 107.0486	Marinoid C	Iridoid glycosides	Leaves/cotyledons/pericarps	IL3	[7]
19	6.65	653.2091	$C_{29}H_{34}O_{17}$	9.55333	621.1822, 459.1499, 179.0338, **161.0234**, 151.0388, 135.0437	Suspensaside methyl ether	Phenylethanoid glycosides	Roots	IL2	[49,50]
20	6.79	623.1981	$C_{29}H_{36}O_{15}$	0.0705	461.1657, **161.0233**, 113.0283	Verbascoside (acteoside) isomer	Phenylethanoid glycosides	Leaves/pericarps/roots	IL2	[52]
21	6.9	463.0874	$C_{21}H_{20}O_{12}$	1.7229	301.0324, **300.0264,** 271.0235, 255.0285	Quercetin 3-*O*-hexoside	Flavonoid glycosides	Roots	IL2	[53–55]
22	7.02	667.2239	$C_{31}H_{40}O_{16}$	0.6864	621.1824, 459.1499, **179.0338**, **161.0235**, 151.0386, 135.0436	β-ethyl-OH-verbascoside	Phenylethanoid glycosides	Pericarps	IL2	[56,57]
23	7.11	623.2001	$C_{29}H_{36}O_{15}$	−3.1336	461.1661, **161.0235**,	Verbascoside (acteoside) isomer	Phenylethanoid glycosides	Leaves/pericarps/roots	IL2	[52]
24	7.21	621.1838	$C_{29}H_{34}O_{15}$	−2.0992	461.1652, 179.0337, **161.0233**, 151.0387	Suspensaside A	Phenylethanoid glycosides	Roots	IL2	[49,50]
25	7.21	461.0718	$C_{21}H_{18}O_{12}$	1.6222	**285.0391**	Kaempferol-3-*O*-glucuronide	Flavonoid glycosides	Leaves	IL2	[58]
26	7.21	681.2063	$C_{31}H_{38}O_{17}$	−3.9236	519.1708, 490.1321, 181.0129, 179.0334, **161.0230**	Unidentified	-	Pericarps		

Table 1. *Cont.*

No.	RT (min)	$[M-H]^-$	Formula	Δ ppm	MS/MS	Name	Class	Part	IL	Ref.
27	7.3	447.0926	$C_{21}H_{20}O_{11}$	1.5287	327.0494, 285.0648, **284.0315**, 255.0288, 227.0338, 151.0013	Kaempferol 3-*O*-glucoside	Flavonoid glycosides	Roots	IL2	[55,59–61]
28	7.40	623.1658	$C_{28}H_{32}O_{16}$	−6.4750	**315.0494**, 314.0421, 300.0258, 299.0187, 271.0234	Isorhamnetin-3-*O*-rutinoside	Flavonoid glycosides	Leaves/pericarps	IL2	[62]
29	7.40	491.0828	$C_{22}H_{20}O_{13}$	0.6385	**315.0499**, 300.0264	Isorhamnetin glucuronide	Flavonoid glycosides	Leaves	IL2	[63]
30	7.51	535.1477	$C_{25}H_{28}O_{13}$	−3.7032	329.1021, **179.0338**, 161.0232, 149.0595, 135.0438	Unidentified	-	Leaves/Pericarps		
31	7.51	477.1036	$C_{22}H_{22}O_{12}$	−5.1250	315.0467, **314.0420**, 285.0392, 271.0236, 257.0441, 243.0286,	Isorhamnetin 7-glucoside	Flavonoid glycosides	Leaves	IL2	[63]
32	7.61	471.1874	$C_{22}H_{32}O_{11}$	−0.4545	287.1273, **263.1278**, 219.1379, 201.1273, 186.1036, 147.1166	Unidentified	-	Pericarps		
33	7.86	519.1143	$C_{24}H_{24}O_{13}$	0.2199	315.0472, **314.0423**, 299.0186, 285.0383, 271.0236, 257.0443, 243.0286	Unidentified	-	Leaves		
34	7.90	553.1556	$C_{25}H_{30}O_{14}$	1.2256	329.1021, **197.0445**, 182.0206, 153.0454, 149.0596, 131.0489,	Marinoid D	Iridoid glycosides	Cotyledons/pericarps/propagules/roots	IL3	[7]
35	7.95	505.1757	$C_{25}H_{29}O_{11}$	0.2000	357.1184, 213.0757, 195.0650, 169.0857, 151.0753, **147.0439**, 125.0596, 113.0230, 107.0487, 103.0539	Marinoid A	Iridoid glycosides	Leaves	IL3	[7]
36	8.03	519.1505	$C_{25}H_{28}O_{12}$	0.5766	313.1072, 295.0961, **163.0388**, 149.0596, 145.0282, 131.0490, 119.0487	Unidentified	-	Leaves/cotyledons/pericarps/roots		
37	8.03	475.0887	$C_{22}H_{20}O_{12}$	−1.0510	300.0589, **299.0554**, 285.0358, **284.0318.**	Diosmetin 7-glucuronide	Flavonoid glycosides	Leaves	IL2	[64]
38	8.24	549.1616	$C_{26}H_{30}O_{13}$	−0.4279	343.1176, 325.1064, **193.0495**, 175.0387, 149.0595, 134.0360, 131.0489	Unidentified	-	Leaves/cotyledons/pericarps/roots		
39	8.36	591.2119	$C_{29}H_{36}O_{13}$	−6.0539	179.0333, **161.0234**, 133.0282, 113.0228	Jionoside C	Phenylethanoid glycosides	Pericarps	IL2	[65]
40	8.62	825.4276	$C_{44}H_{66}O_{16}$	0.2536	**663.3744**, 601.3735	Unknown triterpene saponin	Triterpene saponins	Roots	IL3	[66,67]

Table 1. *Cont.*

No.	RT (min)	$[M-H]^-$	Formula	Δ ppm	MS/MS	Name	Class	Part	IL	Ref.
41	8.70	539.2152	$C_{26}H_{36}O_{12}$	−3.3318	193.0485, 183.1010, 175.0382, 149.0591, 131.0485, 121.0642	Unidentified	-	Leaves		
42	8.80	541.2285	$C_{26}H_{38}O_{12}$	1.0147	193.0485, 185.1166, 175.0382, 149.0591, 131.0485, 121.0642	Unidentified	-	Leaves		
43	8.88	825.4285	$C_{42}H_{66}O_{16}$	−0.8354	**663.3744**, 601.3735, 487.3421	Unknown triterpene saponin	Triterpene saponins	Roots	IL3	[66,67]
44	8.97	299.0546	$C_{16}H_{12}O_6$	5.0379	285.0345, **284.0313**, 256.0363, 227.0334	Trihydroxy-methoxyflavone	Flavones	Leaves	IL2	[68]
45	8.99	825.4273	$C_{42}H_{66}O_{16}$	0.6166	**663.3744**, 601.3735,	Medicoside G (medicagenic acid 3,28-di-glucoside)	Triterpene saponins	Roots	IL2	[66,67]
46	9.08	809.4316	$C_{42}H_{66}O_{15}$	1.5979	689.3884, **647.3788**, 629.3680, 585.3786	Esculentoside C (phycolaccoside D)	Triterpene saponins	Cotyledons/pericarps/propagules/roots	IL2	[69,70]
47	9.30	505.1711	$C_{25}H_{30}O_{11}$	0.8600	281.1170, 195.0649, 151.0750, **147.0438**, 133.0645, 107.0486	Unidentified	-	Leaves		
48	9.38	503.1572	$C_{25}H_{28}O_{11}$	−2.6077	279.1010, 253,0854, 209.0954, 195.0647, **147.0437**, 131.0486, 103.0536	Unidentified	-	Leaves/pericarps		
49	9.54	809.4342	$C_{42}H_{66}O_{15}$	−1.6102	**647.3797**, 471.3469	Azukisaponin III	Triterpene saponins	Roots	IL2	[71]

Analysis detected a total of 49 compounds across all plant parts. Triterpene saponins are a heterogeneous secondary metabolite consisting of a terpene-based aglycone linked to one or more sugar chains, commonly glucose (−162 Da), glucuronic acid (−176 Da), and pentoses (−146 Da) [72]. For example, compound 49, which has an *m*/*z* of 809.4342 and a molecular formula of $C_{42}H_{66}O_{15}$, displayed characteristic MS/MS fragments at *m*/*z* 647.3797 [M-H-162] and 471.3469 [M-H-162-176], corresponding to sequential losses of sugar moieties. Based on this fragmentation and the molecular formula, it was identified as Azukisaponin III. Using similar fragmentation patterns, compounds 40, 43, 45, and 46 were also assigned as triterpene saponins [66,67,69–71].

Phenylethanoid glycosides are often based on β-D-glucosides of 2-phenylethanol, often with α-L-rhamnose (Rha) substitution at C-3′ of the glucose, resembling variants of verbascoside [73]. Simple phenylethanoid glycosides such as acteoside, isoacteoside, and plantamajoside exhibit similar fragmentation patterns in MS/MS experiments. These are characterized by neutral losses of 162, 152, or 146 *m*/*z*, which are associated with the presence of caffeic acid, glucose, rhamnose, and the phenethanol aglycone. Diagnostic fragment ions at *m*/*z* 179, 161, and 135 indicate the presence of caffeoyl, anhydroglucose, and anhydrophenethanol. Additionally, losses of water (−18 Da) or CO_2 (−44 Da) are frequently observed [74]. Based on this information, the compounds 7, 13, 14, 17, 20, 22, 23, 24, and 39 belonged to the phenylethanoid glycosides group.

Flavonoid glycosides are a group of secondary metabolites that are widely distributed throughout the plant kingdom. Depending on the bond of the sugar portion, they are divided into *O*-glycosides or C-glycosides and can be distinguished by their unique MS/MS fragmentation spectra, which depend on the nature of the sugar fraction. Generally, C-glycosides exhibit neutral losses of 30, 90, and 120 Da for hexose sugars; 74 and 104 Da for deoxyhexose sugars; and 60 Da for pentose sugars. In contrast, O-glycosides exhibit neutral losses of 162 Da (hexose sugars), 176 Da (glucuronic acid), 146 Da (deoxyhexose sugars), and 132 Da (pentose sugars) [75]. Based on this information, compounds 12, 21, 25, 27, 28, 29, 31, and 37 were identified as O-glycosides.

Iridoid glycosides exhibit distinct fragmentation patterns that depend on the structure of the aglycone ring, the presence of functional groups, and the degree of unsaturation. Typically, a neutral loss of 162 Da is observed, corresponding to the breakage of the bond with the glucoside fraction. Subsequently, the formation of fragments due to the loss of water (18 Da) and the carboxyl group (44 Da) is also observed, together with characteristic fragments resulting from aglycone ring cleavage. Peak 3 with *m*/*z* 373.1139 and molecular formula $C_{16}H_{22}O_{10}$ was identified as geniposidic acid based on its MS/MS spectrum. Fragment *m*/*z* 211.0605 corresponded to the loss of hexose sugar (162 Da), followed by the presence of fragments *m*/*z* 167.0700 and 149.0597, reflecting subsequent losses of H2O (−18 Da) and CO2 (−44 Da), respectively [76]. Furthermore, fragment 123.0440 is characteristic of the genistein ring. Based on the different fragmentation patterns, compounds 5, 6, 18, 34, and 35 were identified as iridoid glycosides.

Tissue-specific profiling revealed clear metabolic differentiation among parts, with the leaves containing the highest number of secondary metabolites (26), followed by the pericarps (23), roots (23), cotyledons (10), and propagules (6). Notably distinct distribution patterns were observed for specific classes of compounds across the different *A. marina* extracts. The results show that triterpene saponins occur almost exclusively in the root extract (five in roots and only one each in cotyledons, pericarps, and propagules; none in leaves). Phenylethanoid glycosides were predominantly found in root and pericarp extracts (seven in each), with only two detected in leaves and none in cotyledons and propagules. Flavonoid glycosides were mainly associated with leaf extract (six in leaves, two in roots, one in pericarps, and absent in cotyledons and propagules). In contrast, iridoid glycosides and hydroxycinnamic acid and derivatives showed a more uniform distribution across all the extracts.

Analysis confirmed several compounds previously reported in *A. marina*, including caffeoylquinic acid, geniposidic acid, marinoid A, C, and D, acteoside, quercetin 3-*O*-hexoside, kaempferol 3-*O*-glucuronide, isorhamnetin-3-*O*-rutinoside, diosmetin 7-glucuronide, and jionoside C [5,7,77–80]. Additionally, cistanoside F and kaempferol 3-*O*-glucoside were also detected, previously reported in other mangrove species but not in *A. marina* [81,82]. To our knowledge, several compounds such as mussaenosidic acid, (epi)loganic acid, caffeoylglucaric acid, icariside D1, suspensaside, grandifloroside, suspensaside methyl ether, suspensaside A, isorhamnetin glucuronide, isorhamnetin 7-glucoside, medicoside G, esculentoside C, and azukisaponin III have been newly reported in mangrove species.

2.2. Antioxidant Activity

2.2.1. DPPH and ABTS Assays

The antioxidant potential of *A. marina* extracts was evaluated using two spectrophotometric assays, ABTS and DPPH, which are widely used to assess the free radical scavenging activity of natural compounds. The results are shown in Figure 1.

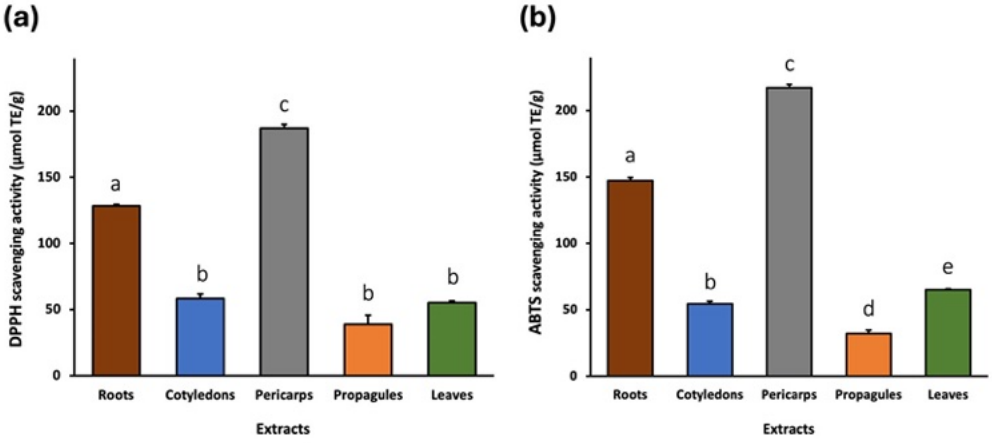

Figure 1. DPPH (**a**) and ABTS (**b**) radical scavenging activity of *A. marina* extracts expressed as µmol Trolox equivalents per gram of sample matrix (µmol TE/g). The bars represent the mean ± standard deviation (SD) from n = 3 independent experiments. Different lowercase letters indicate statistically significant differences between extracts ($p < 0.05$).

The DPPH assay showed that the pericarp extract exhibited the highest radical scavenging activity, with a Trolox equivalent antioxidant capacity (TEAC) value of 187.14 ± 2.87 µmol TE/g. This was followed by the extracts of root (128.25 ± 1.12 µmol TE/g), cotyledon (58.23 ± 3.49 µmol TE/g), leaf (55.12 ± 1.52 µmol TE/g), and propagule (38.72 ± 6.96 µmol TE/g).

Similarly, the ABTS assay confirmed that the root and pericarp extracts exhibit high antioxidant activity compared to the other parts of the plant. In fact, the pericarp extracts again displayed the highest TEAC value (217.16 ± 2.67 µmol TE/g), followed by the root (147.21 ± 2.42 µmol TE/g), leaf (64.98 ± 0.84 µmol TE/g), cotyledon (54.46 ± 1.95 µmol TE/g), and propagule (32.23 ± 2.53 µmol TE/g) extracts.

2.2.2. Correlation Between Compound Classes and Antioxidant Activity

The pericarp and root extracts, which exhibited the highest antioxidant activity, are also the ones that contain a high number of phenylethanoid glycosides, compared to the other extracts, which may explain their higher activity. To explore the potential associations between the phytochemical composition of each extract and their antioxidant capacity, a

Spearman correlation analysis was performed between the number of compounds in each major chemical class and the measured antioxidant activities (DPPH and ABTS assay) across the five plant-part extracts (n = 5) (Table 2). The analysis revealed a significant positive correlation between the number of phenylethanoid glycosides in the extracts and DPPH activity ($\rho = 0.949$; $p = 0.014$). ABTS activity showed a similar trend, though the correlation did not reach statistical significance ($\rho = 0.791$; $p = 0.111$). Antioxidant activity showed no statistically significant correlations with the number of other classes of compounds, including flavonoid glycosides, iridoid glycosides, hydroxycinnamic acids and derivatives, and triterpene saponins (all $p > 0.05$). Given the small sample size and the use of compound counts (not concentrations), these associations should be considered exploratory.

Table 2. Spearman correlation coefficients (ρ) between the number of compounds per chemical class and the antioxidant activity (DPPH and ABTS assays). Statistically significant correlations are indicated in bold (*p* value < 0.05).

Compound Class	**DPPH**		**ABTS**	
	ρ-Value	***p*-Value**	***p*-Value**	***p*-Value**
Iridoid glycosides	−0.103	0.870	0.510	0.935
Hydroxycinnamic acid and derivatives	−0.112	0.858	0.224	0.718
Phenylethanoid glycosides	0.791	0.111	0.949	**0.014**
Flavonoid glycosides	0.205	0.741	0.574	0.322
Triterpene saponins	0.447	0.450	0.224	0.718

2.3. *In Vitro Cytotoxic Activity*

The cytotoxic effects of *A. marina* extracts (leaf, cotyledon, pericarp, propagule, and root) were evaluated against a panel of human cancer cell lines using the MTT assay. Four concentrations (20, 60, 180, and 540 µg/mL) were tested on two colorectal cancer cell lines (SW480 and E705) (Figure 2) and three additional cancer cell lines: MDA-MB-231 (triple-negative breast cancer), U-87 (glioblastoma), and HeLa (cervical cancer) (Figure 3). Furthermore, two non-cancerous cell lines, MRC-5 (normal human fibroblasts) and CCD 841 (healthy human mucosa), served as controls to assess extract selectivity (Figure 4). The complete data for all concentrations and cell lines are reported in Table S1.

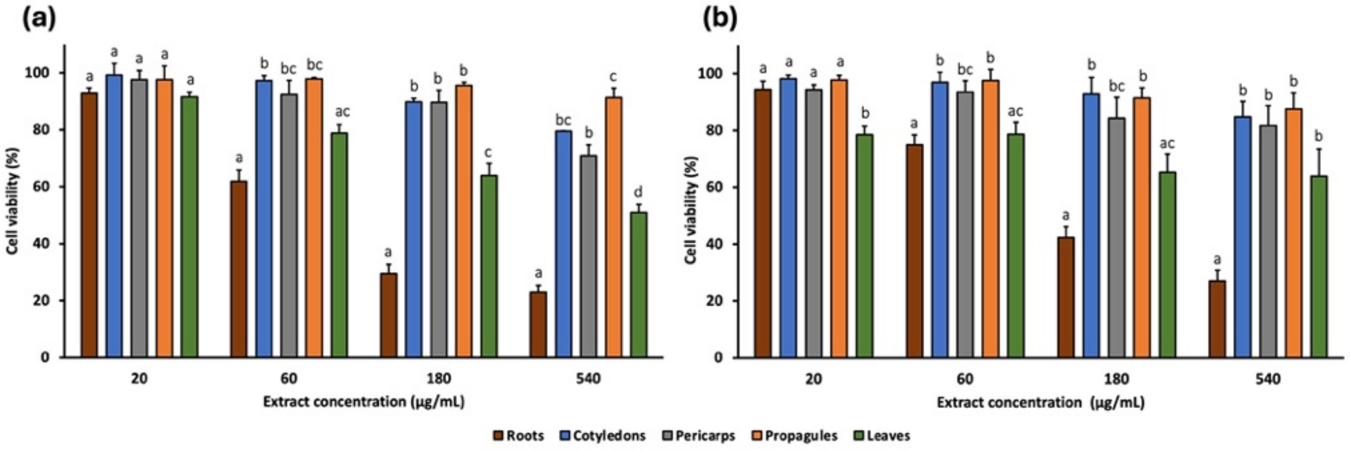

Figure 2. Cell viability (%) of SW480 (**a**) and E705 (**b**) human colorectal cancer cell lines treated with *A. marina* extracts (20–540 µg/mL) for 48 h. Bars represent mean ± standard error of the mean (SEM) from n = 3 independent experiments. Different lowercase letters indicate statistically significant differences between extracts ($p < 0.05$) and were assigned independently for each concentration.

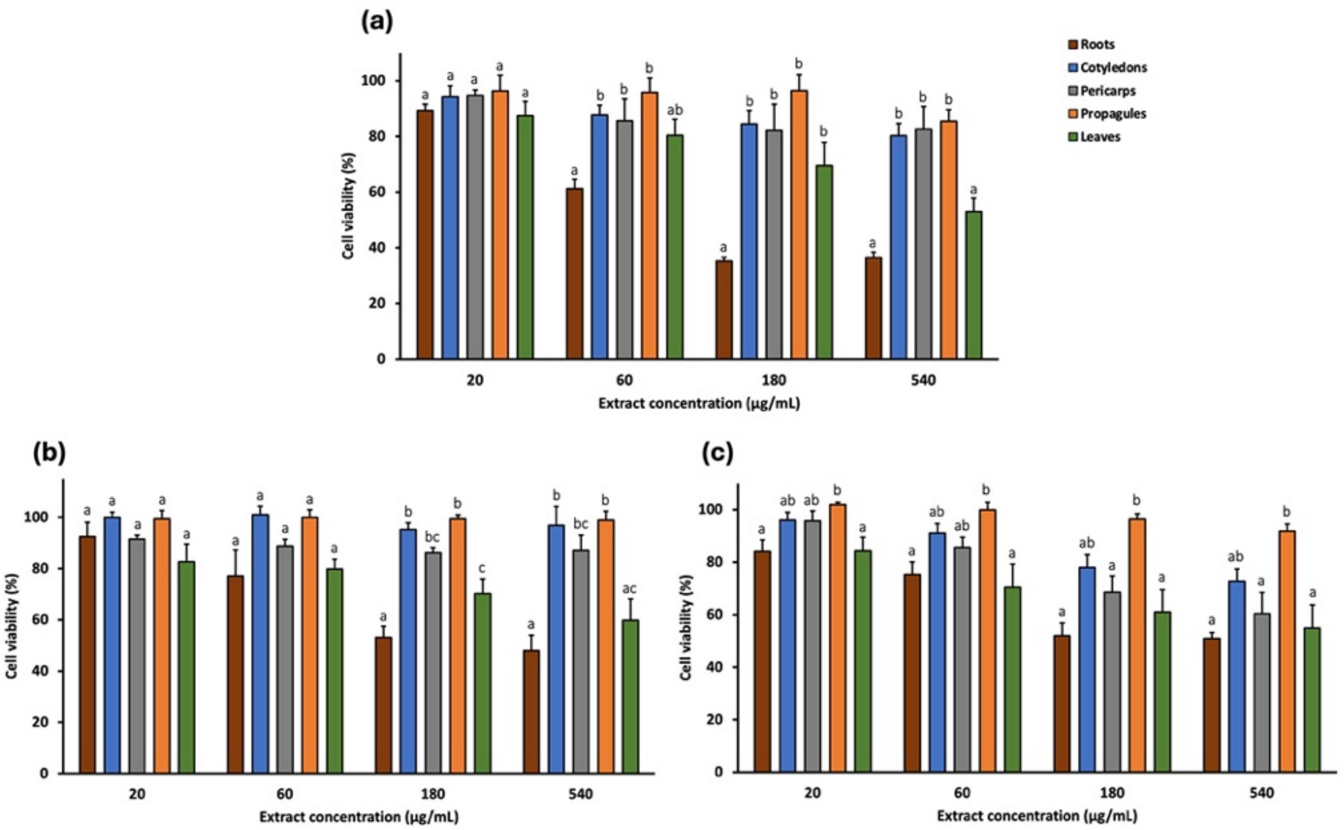

Figure 3. Cell viability (%) of MDA-MB-231 (**a**), U-87 (**b**), and HeLa (**c**) human cancer cell lines treated with *A. marina* extracts (20–540 µg/mL) for 48 h. Bars represent mean ± standard error of the mean (SEM) from n = 3 independent experiments. Different lowercase letters indicate statistically significant differences between extracts ($p < 0.05$) and were assigned independently for each concentration.

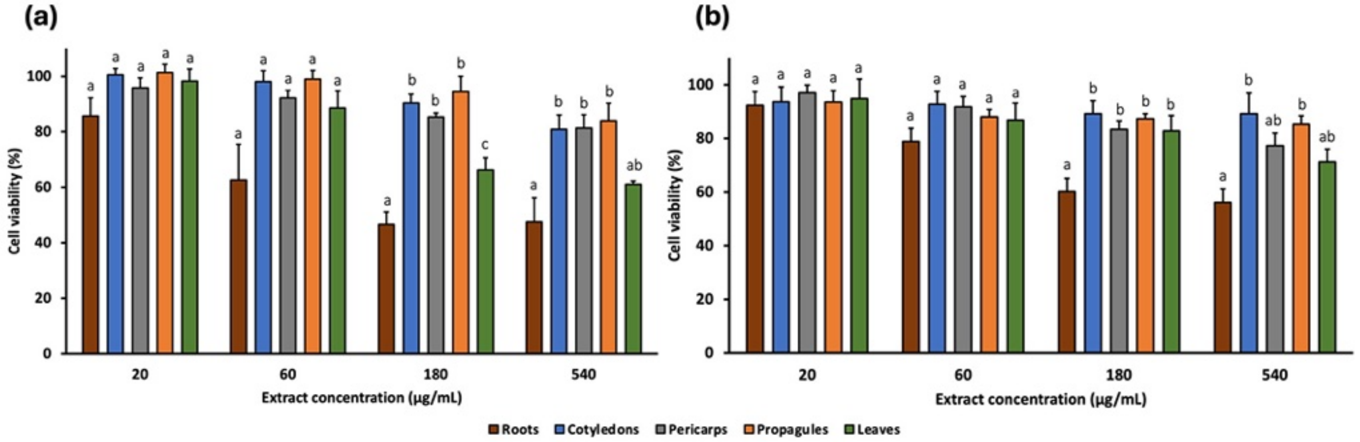

Figure 4. Cell viability (%) of CCD 841 (**a**) and MRC-5 (**b**) healthy human cell lines treated with *A. marina* extracts (20–540 µg/mL) for 48 h. Bars represent mean ± standard error of the mean (SEM) from n = 3 independent experiments. Different lowercase letters indicate statistically significant differences between extracts ($p < 0.05$) and were assigned independently for each concentration.

Among the extracts, cotyledon, pericarp, and propagule generally exhibited the lowest cytotoxic activity, reducing cell viability by no more than 70% at the highest concentration (540 µg/mL) across all cell lines. The exception was the pericarp extract, which reduced viability of HeLa cells to 60.30%.

The leaf extract showed low cytotoxicity at lower concentrations. At 60 µg/mL, cell viability remains near 80% for SW480, E705, and MDA-MB-231 and was 70.44% for HeLa.

In the non-cancerous cell lines, viability was even higher: 88.59% and 86.86% for CCD 841 and MRC-5, respectively. However, at 540 μg/mL, the extract reduced viability to 50–60% in most cell lines, particularly 50.98% for SW480, 63.91% for E705, 53.00% for MDA-MB-231, 59.77% for U-87, 54.96% for HeLa, and 61.01% for CCD 841, while the least reduction occurred in MRC-5 (71.26%).

Among all extracts, the root extract exhibited the highest cytotoxic activity. At 180 μg/mL, it reduced cell viability to 29.47% (SW480), 42.40% (E705), 35.26% (MDA-MB-231), 53.06% (U87), 52.00% (HeLa), 46.60% (CCD 841), and 60.15% (MRC-5). These reductions were statistically significant compared to all other extracts, except for E705, where differences with the leaf extract were not significant, and for HeLa. At 540 μg/mL, cytotoxicity remained similar across most lines, although viability dropped further in SW480 and E705 (22.93% and 27.03%, respectively).

These findings highlight the notable activity of the root extract, particularly at the highest concentrations, against SW480, E705, and MDA-MB-231 cell lines (Figure 5). Notably, at 180 μg/mL, the cytotoxic activity of the root extract in these cell lines was significantly greater than in non-cancerous MRC-5 cells, while no significant difference was observed compared to the healthy mucosa cell line CCD 841. Additionally, at 540 μg/mL, viability of SW480 cells was significantly lower than that of CCD 841.

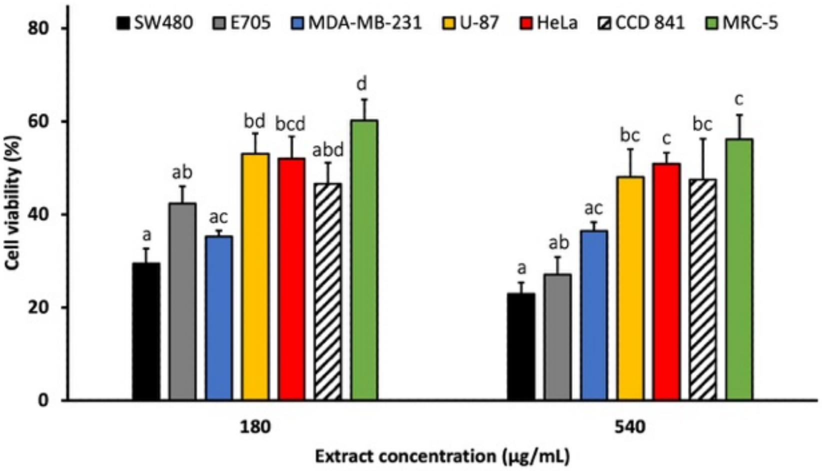

Figure 5. Cell viability (%) of the cancer cell lines treated with *A. marina* root extract (180 and 540 μg/mL) for 48 h. Bars represent mean ± standard error of the mean (SEM) from n = 3 independent experiments. Different lowercase letters indicate statistically significant differences between extracts ($p < 0.05$) and were assigned independently for each concentration.

Given this pronounced response, the analysis focused on dose–response effects in SW480, E705, and MDA-MB-231. Each cell line was treated with ten increasing concentrations of root extract (2 to 540 μg/mL; Figure S6), and the IC_{50} values were 81.98 μg/mL for SW480, 108.10 μg/mL for E705, and 57.93 μg/mL for MDA-MB-231.

2.4. In Silico Analysis

Given the strong cytotoxic activity observed for the *A. marina* root extract in vitro, an in silico analysis was conducted to evaluate the predicted biological activities of the compounds found exclusively or predominantly in this extract. PASS Online predicted potential cytotoxicity-related effects, including antineoplastic activity, apoptosis induction (caspase 3/8 stimulation and apoptosis agonism), TP53 expression enhancement, NF-κB stimulation, cytostatic activity, lipid peroxidase inhibition, and inhibition of ICAM-1 expression [83,84]. The complete list of predicted biological activities for compounds exclusive of the root extract is provided in Table S2 in the Supplementary Materials section.

Among the primary peaks detected in the root extract, the triterpene saponins medicoside G and azukisaponin III were found exclusively in the root extract, while esculentoside C was detected at high levels in the roots and in trace amounts in other extracts. These compounds showed high predicted probabilities (Pa) for antineoplastic activity (0.870, 0.905, and 0.908 for medicoside G, esculentoside C, and azukisaponin III, respectively), caspase 3/8 stimulation (0.994/0.984, 0.989/0.986, and 0.964/0.934), apoptosis agonism (0.901, 0.862, and 0.883), and NF-κB stimulation (0.965, 0.917, and 0.904). They were also predicted to inhibit ICAM-1 expression (0.908, 0.961, and 0.987) and lipid peroxidase activity (0.927, 0.952, and 0.991).

Additional compounds found exclusively in the root extract, including I, suspensaside A, kaempferol 3-O-glucoside, and quercetin 3-O-hexoside, were also predicted to exhibit antineoplastic activity with Pa values of 0.804, 0.863, 0.834, and 0.833, respectively. Kaempferol 3-O-glucoside and quercetin 3-O-hexoside also showed cytostatic activity (Pa: 0.811 and 0.825), enhancement of TP53 expression (Pa: 0.952 and 0.959) and lipid peroxidase inhibition (0.960 and 0.976, resp.).

3. Discussion

UHPLC-ESI/HRMS analyses enabled the characterization of *A. marina* extracts, revealing a total of 49 compounds, unevenly distributed across plant parts. The leaf extract contained the highest number of secondary metabolites, followed by pericarps, roots, cotyledons, and propagules. Notably, this study distinguishes between the external tissue of the propagule (here consistently called pericarp) and the internal tissues of the propagule (here consistently called simply propagule) [85], which are often analyzed as a single fruit unit in other studies. The propagule, which consists mainly of the embryo, contained few compounds, likely due to the focus on primary metabolites essential for germination [86]. In contrast, the pericarp, which serves a protective function, was significantly richer in secondary metabolites [87], particularly phenylethanoid glycosides. Similarly, cotyledons had low metabolite diversity and a profile similar to propagules but showed some additional peaks. These may reflect early biosynthesis of stress-related compounds in developing seedlings.

The compounds identified, including phenylethanoid glycosides, flavonoid glycosides, iridoid glycosides, hydroxycinnamic acid and derivatives, and triterpene saponins, are well known for their ecological roles in protecting plants from abiotic stressors such as drought, high salinity, intense sunlight, and elevated temperatures [33–36,38]. These classes are widely reported in *A. marina* from other regions, and some metabolites identified here have been documented previously, suggesting a shared core phytochemical profile with global populations [10].

However, differences from previous studies were observed. Firstly, a distinctive feature of *A. marina* elsewhere is the presence of naphthalene derivatives [10], which were not detected in our samples. This absence may reflect tissue specificity, as most of these compounds were extracted from branches, or differences in extraction methods [8]. More importantly, several compounds detected in this study have never been reported before in *A. marina*. These include one kaempferol-glycosides and two isorhamnetin glycosides. Notably, monohydroxy B-ring-substituted flavonoid glycosides (e.g., kaempferol-, diosmetin-, and isorhamnetin-glycosides) were more abundant than dihydroxy types, a pattern opposite to that expected under UV stress, where dihydroxy forms typically dominate due to their antioxidant potential [33,35,88]. This shift may reflect Gulf-specific regulation of flavonoid biosynthesis rather than species-specific traits, as it is not evident in previous reports [10]. The study identified novel compounds among the iridoid glycosides and hydroxycinnamic acids, but the most notable findings were within phenylethanoid glycosides and triterpene saponins, the latter all newly reported in *A. marina*. The high abundance of phenylethanoid glycosides in roots is consistent with their reported accumulation under water stress [36], while the accumulation of triterpene saponins is associated with osmotic stress response [38,89]. These trends suggest that UAE-grown *A. marina* may possess a distinctive phytochemical profile shaped by the extreme environmental conditions of the Arabian Gulf [40,90] Nonetheless, inter-regional comparisons are limited due to methodological differences. Future comparative studies using standardized LC-MS protocols would enhance our understanding of mangrove chemical ecology and support bioprospecting efforts.

The identified metabolite classes are associated with various biological activities, including antioxidant, anticancer, antimicrobial, and anti-inflammatory [91–95]. In particular, phenylethanoid glycosides are well-documented antioxidants, showing both DPPH and ABTS radical scavenging activity [91,96,97]. In this study, extracts of the pericarp and root were rich in these compounds and showed the highest antioxidant activity (187.14 ± 2.87 and 217.16 ± 2.67 µmol TE/g for the pericarps; 128.25 ± 1.12 and 147.21 ± 2.42 µmol TE/g for the roots). The strong positive Spearman correlation between the number of phenylethanoid glycosides and DPPH activity ($\rho = 0.949$; $p = 0.014$) supports the hypothesis that these compounds contribute to radical scavenging in the extracts. Key phenylethanoid glycosides identified in this study, such as cistanoside F, acteoside, and jionoside C, are known for their potent antioxidant activity [98–100], while less-studied molecules such as suspensaside and suspensaside A warrant further exploration. Our correlation findings are exploratory and do not prove causation. Definitive attribution will require targeted quantification of candidate phenylethanoid glycosides and subsequent activity testing.

In terms of cytotoxic activity, the cotyledon, pericarp, and propagule extracts showed negligible effects on cancer cell lines, even at the highest concentrations. In contrast, the leaf and root extracts displayed cytotoxicity at higher doses. The leaf extract showed limited cytotoxicity at lower concentrations but reduced viability (50–60%) at 540 µg/mL in several cancer cell lines. This agrees with Momtazi-Borojeni et al. [101], who reported no toxicity at low concentrations but moderate effects at higher doses (250 µg/mL). The root extract had the most promising cytotoxic profile, particularly against SW480, E705, and MDA-MB-231 cancer cell lines. At 540 µg/mL, it reduced cell viability below 40% but showed lower toxicity against normal cell lines (MRC-5 and CCD 841). These findings agree with previous studies showing selective cytotoxicity of root extracts towards cancer cell lines [102]. IC_{50} values for SW480, E705, and MDA-MB-231 were 81.98, 108.10, and 57.93 µg/mL, respectively. Based on the criteria established by the National Cancer Institute (NCI, USA) and the Geran protocol, which classified cytotoxicity as high when IC_{50} values are $\leq$ 20 µg/mL, moderate between 21 and 200 µg/mL, weak between 201 and 500 µg/mL, and absent above 500 µg/mL [103–105], this corresponds to moderate cytotoxic activity. While these IC50 values were not compared with a standard drug, they suggest the presence of active compounds. The values reported here are for crude extracts; further fractionation and isolation of active constituents are expected to yield more potent compounds, for which in vivo and clinical potential could be more realistically assessed through comparison with standard anticancer drugs. These are potentially triterpene saponins, which were only detected in the roots.

Triterpene saponins are gaining attention in cancer research due to their ability to target tumor-related pathways while maintaining low toxicity [106]. Although widespread in medicinal plants [107,108], their occurrence in mangroves is less documented, with only a few studies published on this topic [82,108]. In silico prediction using PASS software indicated a strong cytotoxic potential for several saponins identified in the root extract, including medicoside G, esculentoside C, and azukisaponin III. Additionally, two unidentified saponins suggest the presence of a potentially novel structure that merits further isolation and structural characterization. Other compounds specific to the root extract, such as suspensaside A and kaempferol 3-*O*-glucoside, showed high predicted probabilities for antineoplastic effects.

Given that the root extract exhibited the highest cytotoxicity, further work will focus on bioactivity-guided fractionation of this extract, with particular emphasis on isolating the triterpene saponin-rich fraction. These purified fractions will be tested for cytotoxicity alongside a standard anticancer drug (e.g., doxorubicin) to identify the compounds responsible for the observed activity. Mechanistic studies, including apoptosis assays, cell cycle analysis, and molecular pathway investigations, will be conducted to elucidate the modes of action. Such comprehensive analyses, together with the targeted quantification of candidate constituents, will clarify structure–activity correlations and enhance both therapeutic efficacy and selectivity. Importantly, these efforts, combined with further purification and structural elucidation, could identify promising novel lead compounds suitable for subsequent in vivo evaluation and development as potential anticancer agents.

4. Materials and Methods

4.1. Chemicals

Ethanol absolute, analytical-grade methanol, 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$), and 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\bullet +}$) reagents were obtained from Sigma-Aldrich (Milan, Italy), while methanol and formic acid of LC-MS grade were sourced from Romil (Cambridge, UK). Ultrapure water (18 MΩ) was prepared by a Milli-Q purification system (Millipore, Bedford, MA, USA).

4.2. Plant Material

Samples were collected from different parts of *A. marina*, including leaves, roots, propagules, and cotyledons. The cotyledons were obtained from seedlings at an early growth stage, when the propagules had already opened and developed roots. All samples were harvested in September 2022 from multiple individual plants within the mangrove forest of Ajman Emirate, UAE. Although no herbarium voucher specimen was deposited, the plant material was identified based on morphological characteristics following established taxonomic keys [109]. This identification is supported by the fact that *A. marina* is the only mangrove species forming the evergreen coastal forests of the UAE [14], and its presence in the region has been validated by previous molecular analyses [110,111].

For more precise phytochemical characterization, propagules were separated into the pericarp, representing the external protective tissue, and the internal tissues. Thus, in the manuscript, the term pericarp (and pericarp extract) refers exclusively to the external part, while the generic term propagule (and propagule extract) refers to the internal tissues. Each type of plant material (e.g., all collected leaves, roots, pericarps, propagules, and cotyledons) was pooled by type and immediately freeze-dried after collection. The dried samples were homogenized using a Grindomix GM 200 knife mill (Retsch, Haan, Germany) and then sieved through a test sieve (Retsch AS 200, Haan, Germany) with a mesh size range of 300–600 μm to obtain powders with uniform particle size distribution.

4.3. Sample Preparation and Extraction

Root, leaf, cotyledon, pericarp, and propagule samples of *A. marina* underwent exhaustive ultrasound-assisted extraction using a thermostatically controlled ultrasonic bath (Sonorex TK 52; Bandelin electronic, Berlin, Germany). Each sample was extracted under controlled conditions (25 °C, 15 min) with 50% aqueous ethanol (v/v) at a solid-to-solvent ratio of 1:10 (w/v), which is commonly applied in metabolite profiling studies [112]; specifically, 1 g of powdered sample was mixed with 10 mL of solvent in a 50-mL polypropylene tube. A 50% aqueous ethanol solution was selected as the extraction solvent due to its effectiveness as a green, low-toxicity system, making it well-suited for bioactivity screening [113,114]. Ethanol–water mixtures offer a balanced polarity and are widely recognized for their ability to efficiently extract a broad range of bioactive compounds, particularly polyphenolic metabolites, which are well known for their antioxidant properties [115,116]. Moreover, this solvent was selected to ensure low toxicity in downstream biological assays, in case traces of solvent remain after evaporation, and because non-polar solvents or higher ethanol concentrations could reduce solubility in aqueous assay media, potentially compromising the suitability of the extracts for biological testing.

To ensure complete extraction, the process was repeated three times with fresh solvent. Following each extraction, the mixtures were centrifuged (13,000× *g*, 10 min), and the supernatants underwent filtration through Whatman No. 1 filter paper. The combined extracts were concentrated under pressure at 40 °C using a rotary evaporator to remove ethanol and subsequently lyophilized (Alpha 1-2 LD freeze dryer, Christ, Germany) to obtain dry residues for further analysis.

The extraction yields of *A. marina* were determined by calculating the ratio of the weight of dried extract obtained to the initial weight of dried plant material powder and expressed as a percentage. The yields were 34.23% for roots, 65.02% for cotyledons, 61.76% for pericarps, 38.55% for propagules, and 33.72% for leaves.

4.4. Characterization of Extracts

The chemical characterization of extracts was performed in negative mode using liquid chromatography coupled with electrospray ionization (ESI) and high-resolution mass spectrometry (UPLC-ESI/HRMS). A Waters ACQUITY UPLC system coupled with a Waters Xevo G2-XS QTof Mass Spectrometer (Waters Corp., Milford, MA, USA) was used. The extracts were dissolved in ultrapure water at a concentration of 100 µg/mL, and then 5 µL of each sample was injected into a Biphenyl column (100 mm × 2.1 mm, 2.6 µm; Phenomenex, Torrance, CA, USA). The chromatographic gradient was conducted with solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in methanol), starting with 95% A for 1 min, followed by a linear gradient to 95% B over 10 min, and 4 min of column washing at 95% B. The flow rate was maintained at 0.4 mL/min. The ESI source was operated under the following conditions: electrospray capillary voltage of 1.5 kV, source temperature of 140 °C, and desolvation temperature of 600 °C. MS spectra were acquired in full range mode, covering a mass range of 100–1000 m/z. MS/HRMS analysis was performed using data-dependent scan (DDA), selecting the two most intense ions from the HRMS scan for collision-induced dissociation (CID) with the following conditions: a minimum signal threshold of 500,000, isolation width at 2.0, and normalized collision energy of 30%. Metabolite identification followed the Metabolomics Standards Initiative (MSI) guidelines, which define three confidence levels indicated in the "IL" column of Table 1: Level 1 (IL1): compounds were unequivocally identified by comparison with authentic reference standards (retention time, MS/MS spectrum, and exact mass); Level 2 (IL2): tentative identifications were assigned based on matches between experimental MS/MS spectra and literature data or spectral libraries (e.g., GNPS, MassBank); Level 3 (IL3): compounds were classified by spectral similarity to known chemical families and supported by taxonomic evidence.

Novelty verification was performed using general literature databases (e.g., Google Scholar) and the chemical database SciFinder^{n}. In SciFinder^{n}, each tentatively identified compound was queried by chemical name, and all related references were investigated using keywords such as "*Avicennia marina*", "*Avicennia*", and "mangroves". This process allowed determination of whether a compound had been previously reported in *A. marina*, other species within the genus *Avicennia*, or other mangrove species, or if it represents a first identification in mangroves.

4.5. Determination of Antioxidant Activity

The antioxidant capacities (AOCs) of the exhaustive extracts of *A. marina* (leaves, roots, pericarps, propagules, and cotyledons) were evaluated using 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) and 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate ($ABTS^{\bullet+}$) assays according to Cannavacciuolo et al. [117]. The extracts were dissolved in ultrapure water and analyzed at a concentration of 0.5 mg/mL, with Trolox (0–500 µM) serving as a standard. The antioxidant activity was expressed as µmol Trolox equivalents per gram of sample matrix (TE/g MTX), representing the µmol of a standard Trolox solution exerting the same antioxidant capacity as 1 mg/mL of the tested extracts.

In the DPPH assay the stock solution of DPPH (5 mM) was prepared by dissolving 3.9 mg of DPPH in 100 mL of methanol and subsequently diluted to 100 µM to obtain the operating solution. This solution was prepared just before use and protected from light due to the photosensitivity of the reagent. The assay was set up in an Eppendorf by mixing 50 µL of sample with 950 µL of operative DPPH, and the mixture was incubated in the dark for 30 min. Subsequently, 200 µL of the solution was transferred to an absorbance reading plate at the 515 nm wavelength.

For the ABTS assay, the stock solution of ABTS (7 mM) was diluted with phosphate-buffered saline (PBS; 5 mM, pH 7.4) to achieve working concentrations. The assay was performed by combining 5 µL of diluted sample (or PBS control) and 500 µL of ABTS radical cation solution (0.1 mM in PBS). The reaction mixtures were protected from light and incubated at 30 °C for 60 min to allow complete radical scavenging. Absorbance measurements were then recorded at 734 nm using a microplate reader.

4.6. Cytotoxicity Evaluation

4.6.1. Cell Lines and Culture Conditions

Normal human fibroblasts (MRC-5), human glioblastoma (U-87), human triple-negative breast cancer (MDA-MB-231), human colorectal cancer (SW480), and human healthy mucosa (CCD841) cell lines were purchased from the American Type Culture Collection (Manassas, VA, USA). The human cervical cancer cell line (HeLa) was acquired from System Biosciences, and the human colon cancer cell line (E705) was provided by the Fondazione IRCCS Istituto Nazionale dei Tumori (Milan, Italy). The E705 cell line represents epithelial tissue cells of colorectal adenocarcinoma derived from a patient at the National Cancer Institute in Milan.

MRC-5, U-87, and HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) high glucose medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin/streptomycin (P/S), and 2 mM L-glutamine. MDA-MB-231 cells were maintained in Minimum Essential Medium (MEM) with Earl's Salts supplemented with 10% heat-inactivated FBS, 1% P/S, 2 mM L-glutamine, and 0.1 mM MEM Non-Essential Amino Acids (MEM NEAA). E705 and SW480 cell lines were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamine. The CCD 841 cell line was grown in EMEM medium supplemented with 10% heat-inactivated FBS, 1% P/S, 2 mM L-glutamine, and 0.1 mM non-essential amino acids. All cell lines were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air. Cell culture media and reagents were purchased from EuroClone (Pero, Italy).

4.6.2. Viability Assay

The cytotoxicity of *A. marina* extracts was evaluated using the MTT assay (CellTiter96® Non-Radioactive Cell Proliferation Assay, Promega, Madison, WI, USA) following the manufacturer's protocol. The extract powders were solubilized in Milli-Q water, and four extract concentrations (20, 60, 180, and 540 µg/mL) were tested on all cell lines. Additionally, for the root extract, an extended dose-response analysis using ten concentrations (2, 10, 20, 40, 60, 100, 140, 180, 360, and 540 µg/mL) was performed on selected cell lines to enable IC_{50} determination. IC_{50} values were calculated only for extract–cell line combinations tested with this ten-point dilution series.

No positive control drugs were included in this preliminary screening, as the primary objective was to assess and compare the relative cytotoxicity of different *A. marina* extracts. Comparative analysis with standard anticancer agents will be incorporated in subsequent studies on purified fractions or isolated compounds.

Briefly, HeLa, MRC-5, U-87, and MDA-MB-231 cells were seeded into 96-well plates (from Euroclone, Pero, Italy) at a density of 5×10^3 cells/well in 100 µL of growth medium, while E-705 and SW-480 cells were seeded at a density of 8×10^3 cells/well. After 24 h of incubation at 37 °C in 5% CO_2, the medium was replaced, and cells were treated with various concentrations of *A. marina* extracts. Following 48 h of treatment, the medium was replaced, and 15 µL of MTT solution was added to each well. After 3 h of incubation at 37 °C, formazan crystals were solubilized using 100 µL of stop solution and incubated under stirring for 1 h. Reduced MTT was quantified using a UV–vis plate reader (EnSight Multimode Microplate Reader, PerkinElmer, Waltham, MA, USA) at 570 nm with a reference wavelength of 630 nm.

Cell viability was expressed as a percentage relative to untreated cells (negative control), and medium with MilliQ water at equivalent concentrations (10% *v*/*v*) was used as a blank. Dose-response curves and the IC_{50} values, representing the extract concentration required to inhibit 50% of cell viability relative to untreated control cells, were generated using GraphPad Prism v10.5.0 software.

4.7. In Silico Prediction for Anticancer Activity

To identify potential bioactive compounds responsible for the cytotoxic effect, an in silico prediction of biological activity was performed using PASS Online software (https://www.way2drug.com/PASSOnline/index.php; accessed on 15 July 2025), a predictive tool from Way2Drug Services. The reliability of PASS for predicting in vitro cytotoxic activity has been demonstrated in previous studies [118,119], including those focusing on triterpene saponins [120].

The canonical Simplified Molecular Input Line Entry System (SMILES) of each compound was gathered from $\text{SciFinder}_\text{n}$ and was used to run the software. The program independently calculates the estimated predictive biological activities based on structure–activity relationships, providing Pa (probability of activity) and Pi (probability of inactivity) values for each activity. Only activities with $Pa > 0.7$ were considered, as this threshold indicates a high likelihood that the substance will exhibit the predicted activity in experimental settings, although the probability of the compound being an analogue of a known pharmaceutical agent remains high [121]. Notably, when $Pa > 0.9$, as frequently observed in our study, the likelihood of false-positive predictions is insignificant [118].

The predicted anticancer-related activities included antineoplastic activity, apoptosis-related effects (apoptosis agonist, caspase 3/8 stimulation), TP53 expression enhancement, NF-kB modulation, cytostatic activity, lipid peroxidase inhibition, and ICAM-1 expression inhibition. These results were analyzed in relation to the cytotoxicity data of the MTT assay to establish potential correlations between the phytochemical composition and the observed cytotoxicity against cancer cells.

4.8. Statistical Analysis

Statistical analyses were conducted on data generated from three replicates. Cytotoxic activity results are presented as mean ± standard error of the mean (SEM). Antioxidant activity results are reported as mean ± standard deviation (SD). Before statistical analysis, the assumption of normality was assessed using the Shapiro–Wilk test. The homogeneity of the variances was evaluated using Levene's test. When normality and homogeneity assumptions were met, a one-way analysis of variance (ANOVA) was performed, followed by Tukey's honest significant difference (HSD) post hoc test to assess pairwise differences between the means of the group. In cases where the assumption of homogeneity of the variances was not met, Welch's ANOVA was applied, followed by the Games–Howell post hoc test. Statistical significance was considered when $p < 0.05$.

The correlation between phytochemical composition and antioxidant activity was assessed using Spearman's rank correlation coefficient (two-tailed). For each plant-part extract ($n = 5$), the number of tentatively identified compounds in each major chemical class (phenylethanoid glycosides, flavonoid glycosides, iridoid glycosides, hydroxycinnamic acids and derivatives, and triterpene saponins) was correlated with antioxidant activity (DPPH and ABTS, mean values). Statistical significance was set at $p < 0.05$.

All analyses were conducted with IBM SPSS Statistics v29.0.2.0.

5. Conclusions

The findings of this study highlight *A. marina* as a valuable source of bioactive compounds with promising therapeutic applications. The pericarp and root extracts exhibited the highest antioxidant activity, possibly due to the presence of phenylethanoid glycosides, which are known for their antioxidant activities. Among all extracts tested, the root extract displayed the strongest cytotoxicity, in particular against the triple-negative breast cancer cell line MDA-MB-231 and two colorectal cancer cell lines, SW480 and E705, with IC_{50} values of 58.46, 81.98, and 108.10 µg/mL, respectively. In silico predictions identified triterpene saponins, including medicoside G, esculentoside C, and azukisaponin III, as likely contributors to these effects.

The detection of triterpene saponins not previously reported from mangroves, together with several phenylethanoid glycosides and other compounds not earlier described in *A. marina*, is noteworthy. Plants adapted to extreme environments can accumulate distinctive secondary metabolites, and our plant-part-specific UPLC-HRMS analysis of UAE-grown *A. marina* provides region-specific evidence that complements existing phytochemical surveys. Moreover, combining this untargeted phytochemical investigation with biological activity screening and in silico analysis/statistical correlation offers a practicable approach to rapidly link observed activities to plant-part-specific compounds.

To advance these observations towards pharmacological relevance, future work should focus on bioactivity-guided fractionation of the extracts, structural elucidation, and targeted quantification of key compounds, along with mechanistic in vitro assays. These efforts will help clarify structure–activity correlations and potentially lead to the identification of a novel therapeutic candidate from this stress-adapted mangrove species.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ph18091308/s1, Figures S1–S5: Representative chromatograms of *A. marina* extracts; Table S1: Cytotoxicity of *Avicennia marina* extracts on the tested cell lines at four concentrations (20–540 µg/mL); Figure S6: Cell viability of SW480 (a), E705 (b), and MDA-MB-231 (c) human cancer cell lines treated with root extract (2–540 µg/mL) for 48 h; Table S2: Probable cytotoxicity-related biological activities of 6 compounds tentatively identified in the root extract of *A. marina* by PASS (Prediction of Activity Spectra for Substances).

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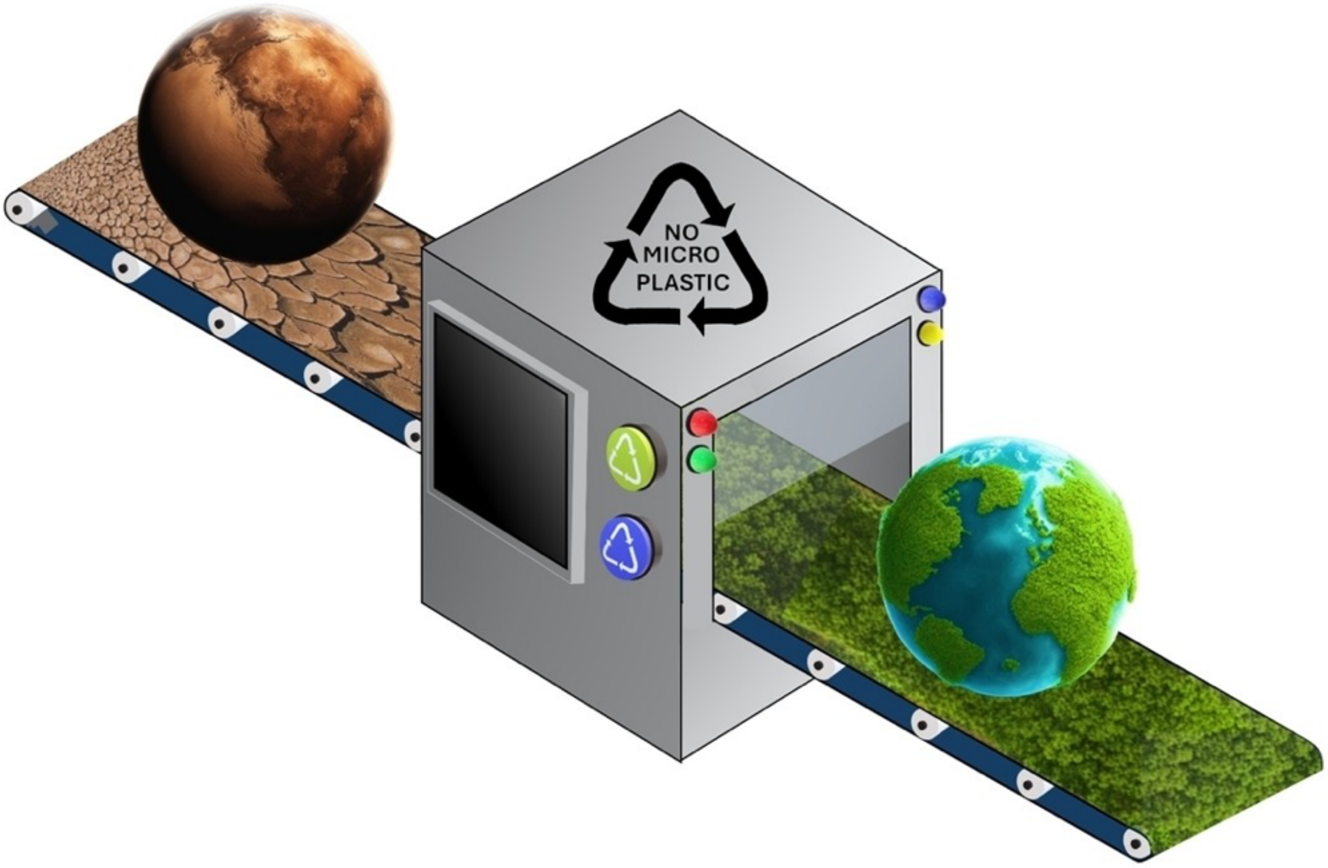
Microplastics in Cosmetics: Open Questions and Sustainable Opportunities

Marco Giustra,[a, b] Giulia Sinesi,[a] Francesca Spena,[a] Beatrice De Santes,[a] Lucia Morelli,[a] Linda Barbieri,[a] Stefania Garbujo,[a, b] Paolo Galli,[c, d, e] Davide Prosperi,*[a, b] and Miriam Colombo*[a, b]



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The cosmetic industry is now changing or rather having an ecological transition in which formulations such as creams, lotions, and powders for make-up, skin and hair care must not contain microplastics, now a taboo word in this field. Nowadays, many companies are intensifying their research and development (R&D) work to align with recent and future legislation that provides for their elimination to safeguard the ecosystem. The production of new eco-sustainable materials is currently a hot topic which finds its place in a market worth above 350 billion dollars which will reach more than 700 billion dollars in a very short time. This review offers an overview of the main advantages and adverse issues relating to the use of microplastics in cosmetics and of their impact, providing an insight into the properties of the polymeric materials that are currently exploited to improve the sensorial characteristics of cosmetic products. In addition, the various regulatory restrictions in the different geographical areas of the world are also described, which is matter for reflection on future direction. Finally, a prospective vision of possible solutions to replace microplastics with sustainable alternatives complete the picture of the next generation personal care products to support decision-making in the cosmetic marketplace.

1. Introduction

Cosmetics is a rapidly expanding economic and scientific sector, as also witnessed by the increasing number of brands and new products entering the marketplace every year, with the search for new materials and new effects capable of responding to new claims.[1]

Cosmetics play a significant role in the daily lives of people worldwide. The interest for their usage is no longer restricted to a concept of vanity and luxury. Indeed, psychological-social factors. Remarkably contribute to such constantly growing attractiveness for cosmetics. In fact, the daily consumption of these products has become a way to express and take care of oneself and increase self-esteem.[2,3] On this basis, the market is always evolving reflecting consumer needs.

Based on the data reported by Fortune Business Insights Pvt. Ltd., the global cosmetics market was estimated at $374.18 billion in 2023 and is projected to exceed $750 billion by 2032, exhibiting a Compound Annual Growth Rate (CAGR) of 9.8%. Notwithstanding the startling numbers, researchers and activists are still conducting studies on the problematic presence of microplastics in cosmetics highlighting their potential risks to the environment. Prior to the advent of microplastics, natural compounds have been used in cosmetic manufacturing since ancient times. Raw materials such as sugar, cellulose, starch, proteins, natural fats, and oils were processed by the chemical industry in various fields. For instance, some vegetable oils such as avocado, lauric, palm, soybean, and walnut were used. Among vegetable fats were hydrogenated vegetable oil and shea butter. Animal sources of fatty acids included lard and tallow.[4,5] Additionally, lanolin alcohols, derived from the fat of wool shearing, were introduced to the market as emollients after the Second World War.[6]

In the ′80s, microplastics replaced these natural ingredients in many products, both in formulations and in the packaging of cosmetic products, due to their versatility. They were used for various functions, including viscosity regulation, film forming, opacifying, bulking, and exfoliating.[7] For example, polyethylene glycols (PEGs) were used as humectants and moisturizers,[8] polyacrylates as film-forming agents,[9] and polyamides to lend substantivity to hair and skin.[10]

However, the first bans regarding marine litter began with the increase in plastic pollution. Countries and organizations have become more aware of the negative impact of plastic and microplastics on the environment. In 2015, the “Microbead-Free Waters Act” was the first ban on microbeads. Subsequently, prohibitions or restrictions on microbeads or microplastics in cosmetic and personal care products were implemented.

Beat the Microbead (http://www.beatthemicrobead.org) was one of the first campaigns published on April 2022 to put under the spotlight the problem of microplastics in cosmetics by promoting broad awareness on this issue to encourage consumers to choose products free of microplastics. *Greenpeace* and *Friends of the Earth* have also helped highlight the issue of microplastics in cosmetics through alertness campaigns, reports, and studies.[11] These efforts are encouraging institutions worldwide to fight against another widespread mystification, which offers new products dishonestly declared to be environmentally friendly – the so-called greenwashing. In 1986, Jay Westerveld coined the term “greenwashing” concerning the idea of encouraging the reuse of towels in hotels suggesting environmental care but actually done as a strategy to reduce laundry costs.[12] Although the concept is not yet formally defined, today this term is adopted to describe practices in which commitment to environmental sustainability is promoted, but all is done primarily for economic and marketing purposes.

The EU defines greenwashing as “*the practice whereby companies claim to be doing more for the Environment than they actually are*”. In 2024, the European Parliament recognized its

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[a] *NanoBioLab, Department of Biotechnology and Bioscience, University of Milano-Bicocca, Milano, Italy*

[b] *Nanobiotechnologies for Health Center, NANOMIB, University of Milano-Bicocca, Vedano al Lambro, MB, Italy*

[c] *Department of Earth and Environmental Sciences, University of Milano-Bicocca, Milano, Italy*

[d] *Dubai Business School, University of Dubai, United Arab Emirates Goumbook, Ras Al Khaimah, United Arab Emirates*

[e] *MaRHE Centre (Marine Research and High Education Center), Magoodhoo Island, Maldives*

Correspondence: *Davide Prosperi and Miriam Colombo, NanoBioLab, Department of Biotechnology and Bioscience, University of Milano-Bicocca, Piazza della Scienza, 2, 20126 Milano, Italy.*
Email: davide.prosperi@unimib.it and miriam.colombo@unimib.it

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provisional agreement with the Council on the Directive Empowering Consumers for the Green Transition through Better Protection against Unfair Practices and Better Information ("Greenwashing Directive").[13] Nevertheless, a specific EU regulation focused on greenwashing in cosmetics does not exist, while just regulations and initiatives to ensure transparency and prevent deceptive marketing practices are being taken.

Over time, consumers have become increasingly aware of the above issue, seeking out products with specific labels such as Cruelty-Free, Vegan, Nickel Tested, Dermatologically & Ophthalmologically Tested, and free of Ethyl Alcohol, Silicone, and Petrochemicals. Additionally, consumers are increasingly choosing products with eco-sustainable packaging and/or made from recycled materials (Green Packaging).[14] In this review, we will focus on a fundamental component in the production of solid and semi-solid formulations: cosmetic powders. Cosmetic powders have been and continue to be at the forefront of sustainability concerns, leading various institutions to enact legislation addressing the issue.

Cosmetic powders are widely used in cosmetic formulations owing to their versatility, ability to enhance sensorial characteristics and skin benefits making the final products appreciated by the consumers. The main features of cosmetic powders include (Figure 1):

- Exfoliating: powders are able to remove dead cells on the skin surface promoting cell renewal and favouring smoother skin.
- Fixative: powders avoid smudging in semi-solid formulations and prolong their shelf life.
- Mattifying or brightening: powders can be used to reduce undesirable reflections on the skin or enhance brightness to the face, especially in the T-zone (forehead, nose and chin), where oiliness tends to accumulate more.

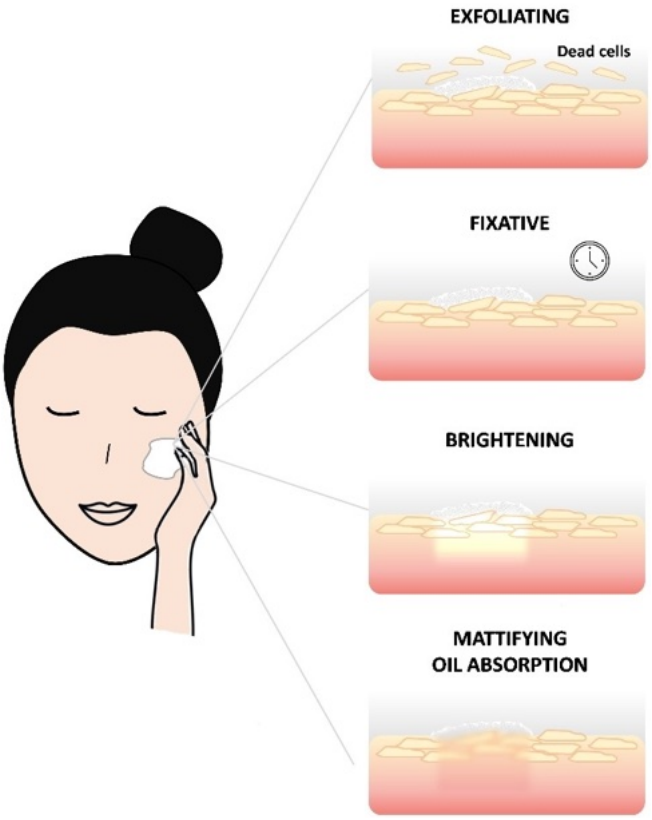

Figure 1. Main characteristics of cosmetic powders in formulation and relative effects on skin: exfoliating, fixative, brightening, mattifying and oil absorption.

- Oil absorption: sebum-normalizing products, some powders help to control oiliness maintaining an opaque and matte appearance.

Marco Giustra graduated in Chemistry from the University of Milano-Bicocca in 2018. He obtained a PhD in Nanotechnology and Materials Science in 2022, focusing on synthesizing inorganic nanoparticles and multi-branching polymers to enhance colloidal stability. His current research is centered on producing sustainable raw materials for cosmetic and pharmaceutical formulations, as well as developing alternative RNA nanodelivery methods for cancer treatment.

Davide Prosperi graduated in Chemistry in 1998 and obtained a PhD in Chemistry in 2002 at the University of Milan. Since 2019, he is Full Professor in Biochemistry at the University of Milan-Bicocca. He is Director of the Nanobiotechnologies for Health Center. His research is mainly focused on colloidal and biomimetic nanoparticles for use as drug delivery systems, molecular imaging and probes for the investigation of intracellular molecular mechanisms, including gene silencing and cell transduction and signaling. Current research topics in his laboratory are RNA therapy for cancer treatment and the development of sustainable materials for nanomedicine and cosmetics.

Prof. Miriam Colombo obtained her Master degree in 2008 in Medicinal Chemistry and Technology at the University of Milano and she made the PhD in Biology in 2012. She is full professor of Clinical Biochemistry at the University of Milano-Bicocca. Her scientific research focuses on developing nanoparticles for biomedical applications. This involves creating new systems for drug delivery and other therapeutic approaches, characterizing them, and bio-functionalizing them with various active bio-ligands. Additionally, she has explored alternative administration methods for nanoparticles and biomolecules, such as oral and topical administration, particularly for pharmaceutical and cosmetic applications.

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These features can be combined to produce formulations capable of satisfying multiple needs, such as setting makeup, controlling skin oiliness, sculpting, and defining facial features.

Actives encapsulation or surface modification of cosmetic powders boost the final product's applicability and performance, such as wetting and anti-ageing effects.

Microplastics have often addressed the main appearances stated, presenting a global and pressing challenge (Figure 2). Currently, some R&D companies, alongside universities are striving to replace microplastics in cosmetic products while maintaining or even improving the powder performance, thus obtaining sustainable products.

2. Microplastics in Cosmetic Formulations – A Real Threat for the Environment and Human Health

In the last decade, microplastics (MPs) have emerged as a significant environmental and health problem worldwide. MPs are defined as small plastic particles ranging in size from 0.1 to 5 mm.[15] Microplastics derive from a variety of sources, including discarded plastic, the textile, and cosmetic industries. The generation of plastics and microplastics associated with the cosmetic industry is currently one of the most serious environmental issues, reinforced also by the increased number of consumers and the high demand in this sector.[16]

Microplastics can be found in primary form, as constituents of cosmetic formulations, such as exfoliating agents, or as secondary plastics, derived from cosmetic packaging. Among the primary sources, personal care products, including cleansing products, makeup cosmetics, shower gel, facial cleanser, hand sanitizer, soap, toothpaste, sunscreen and shampoo represent significant sources of microplastics in the environment.[17] These microplastics are specially designed and optimized in a spherical shape, to reduce skin scratches or disruption of the natural skin growth process. The use of microplastics in various personal care products has expanded beyond their initial scrubbing effects to include important functions such as binders, bulking agents, emulsifiers, film formers, viscosity regulators, opacifying agents, glitters, skin conditioning, tooth polishing in oral care, gellants in denture adhesives, moisturizers, sun filters, and stabilizers.[18]

The plastic ingredients used for the scrubbing function are commonly referred to as plastic microbeads. Microbeads made of polyethylene are the most common application, although polyurethane, polypropylene, polyethylene terephthalate, polymethyl methacrylate and nylon are used.[19] The advantages of using plastic microspheres for washing and exfoliation include their soft peeling effect, associated with good skin tolerance. They have a broad range of positive properties, they are chemically inert substances, odourless, non-sensitizing and non-irritating.[20]

Microbeads have the potential to improve the performance of a wide range of cosmetic products, but there are also some drawbacks to consider. In fact, microbeads, being tiny plastic particles, are difficult to filter out in wastewater treatment plants, entering water streams and eventually contaminating rivers.[21] They can be found in the ocean, soil, and even in the air we breathe.

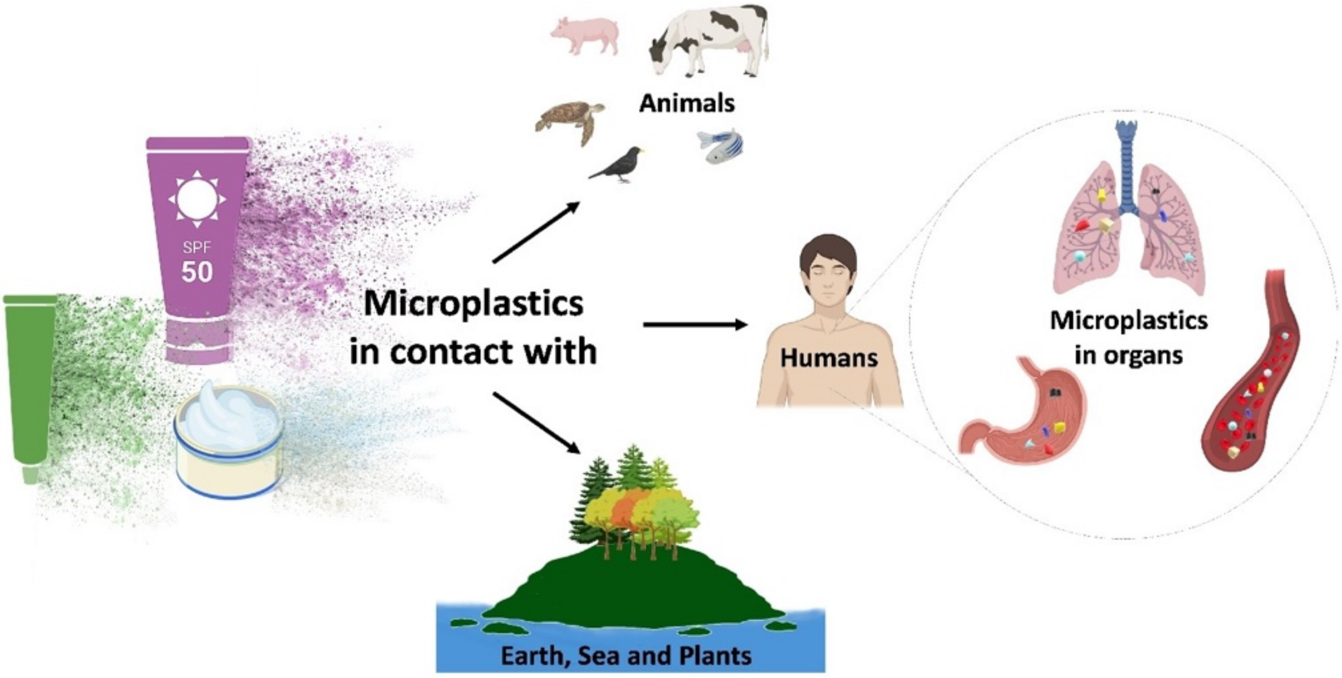

Figure 2. Microplastics from personal care and cosmetic products have the potential to infiltrate the entire ecosystem–reaching the earth, seas, plants, animals, and ultimately, humans. Especially, microplastics reach human organs.

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Since there is no effective way to remove microplastic contaminants from the marine environment and they are highly resistant to degradation, microparticles can be adsorbed or ingested by many marine organisms. The introduction of these microparticles by marine animals, plankton, and other biota results in a negative effect on the entire food chain of the marine ecosystem. The presence of microplastics in different marine organisms, such as copepods,[22] bivalves,[23] fish,[24] and seabirds[25] has already been documented.

Another issue to be considered is that microplastics have large specific surface areas, making them easier to absorb hydrophobic chemicals (e.g., polychlorinated biphenyls) from aquatic environments. They can be considered as vectors for various harmful contaminants, such as heavy metals (Al, Cd, Co, Cr, Cu, Hg, Mn and Pb), polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pesticides and persistent organic pollutants.[26,27]

Furthermore, particles of less than 130 mm, can accumulate in human tissue and diffused into bloodstream, lymphatic system, and organs through various mechanisms, such as ingestion, inhalation, and dermal absorption (Table 1). The implications for human health are substantial and multifaceted, as MPs may release the mentioned toxins, additives and monomers, which may have carcinogenic activity and trigger inflammatory responses.[28] In light of these considerations, there is obviously a need for consolidation of actions, changes and optimization in the production processes and products of the cosmetic industry.

2.1. Human Blood

A study by Leslie et al. provided the first evidence of plastic particles in the bloodstream. They quantified MPs in whole blood samples from 22 healthy individuals,[29] obtaining data for concentrations in blood for five polymers: poly (methyl methacrylate) (PMMA), polypropylene (PP), materials containing polystyrene (PS), polyethylene (PE) and polyethylene terephthalate (PET). PET was the most frequently detected polymer, with measurable values found in 50% of all tested donors. PMMA, PE, and PS were detected in 5%, 23%, and 36% of donors, respectively. The average polymer concentration in blood was 1.6 μg/mL. The study highlighted that plastic particles can enter the human body and be eliminated through the biliary tract, the kidneys or other organs at a slower rate than they are absorbed into the blood. Given the lack of information on the long-term health effects of MPs in human blood, further research is essential to better understand the risks. This involves investigating the sources and pathways by which MPs enter the bloodstream, analyzing their distribution and accumulation patterns, and assessing their potential impacts on various physiological and cardiovascular systems.

More recently, Yang et al. conducted a study which identified MPs in the human heart for the first time.[30] In this study, the presence of MPs in the human heart and its surrounding tissues was investigated by collecting blood venous samples from 15 cardiac surgery patients through a laser direct infrared chemical imaging system and scanning electron microscopy. The most common MPs were polyamide (49%) and PET (22%), making up over 70% of the total microplastic content. The composition of MPs changed significantly before and after surgery. Pre-surgery samples were dominated by PET (67%), whereas post-surgery samples were primarily polyamide (57%). The diameter of MPs also shifted, with pre-surgery MPs mostly between 30 and 50 μm and post-surgery MPs predominantly between 20 and 30 μm. These findings indicate the potential interactions between medical procedures and MP exposure, which can impact on post-operative recovery and cardiovascular health.

Table 1. Predominant microplastics found in human organs and fluids.

Organs and fluids	Detection technique	Particle types	References
Blood	Py-GC/MS	PE, PET, PMMA, PP and PS	Leslie et al.[29]
	LD-IR and SEM	PA, PC, PE, PET, PMMA, PP, PS, PU, and PVC	Yang et al.[30]
Vein tissue	μFTIR	Alkyd resin, Nylon EVA, PVAc, PVAE and PUR	Rotchell et al.[31]
Semen	μRaman	PC, PE, PET, POM, PP, PS, and PVC	Montano et al.[32]
	Py-GC/MS and LD-IR	PA, PE, PET, PP, PS, and PVC	Zhao et al.[33]
Testis	Py-GC/MS and LD-IR	PE, PP, PS and PVC	Zhao et al.[33]
Placenta	μRaman	PP and other fragments	Ragusa et al.[34]
	FTIR	PE, PP and PU	Braun et al.[35]
	VP-SEM and TEM	Fragments compatible with MPs	Ragusa et al.[36]
	LD-IR	PP and PVC	Zhu et al.[37]
	LD-IR	PA and PU	Liu et al.[38]
Pulmunary tissue	μRaman	PE and PP	Amato-Laurenço et al.[39]
Sputum	LD-IR and μFTIR	Alkyd varnish, CPE, PES and PU,	Huang et al.[40]
Urine and kidneys	μRaman	PE, PP, PVA and PVC	Pironti et al.[41]
	μRaman	PE and PS	Massardo et al.[42]

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MPs have also been found in human vein tissue. Rotchell et al. discovered MPs in four out of five vein samples by using µFTIR. The MPs were mainly fragments ranging in size from 16 to 1074 µm. The most common polymers were alkyd resin, polyvinyl propionate/acetate (PVAc), and a tie layer of nylon EVA or ethylene vinyl alcohol (EVOH)-EVA.[31]

2.2. Testis and Semen

Montano et al. analyzed semen samples from men living in a polluted area of Southern Italy to determine the presence of MPs. Spherical and irregular microplastic fragments, ranging in size from 2 to 6 µm, were found in six out of ten samples.[32] Furthermore, the chemical composition analysis identified the presence of commonly used polymers including PP, PS, PET, PE, polyoxymethylene (POM), polyvinylchloride (PVC), and polycarbonate (PC).

In another study by Zhao et al., MPs were discovered in human testis and semen. 6 testis and 30 semen samples were used to detect MPs through pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) and laser direct infrared spectroscopy (LD-IR).[33] MPs were found in both testis and semen, with an average concentration of 0.23 ± 0.45 particles/mL in semen and 11.60 ± 15.52 particles/g in testis. The MPs in the testis were predominantly composed of PS at 67.7 %, while PE and PVC were the main polymers in semen. The MPs were between 21.76 µm and 286.71 µm in size, with the majority (67 % in semen and 80.6 % in testis) being between 20 and 100 µm. This study is the first to reveal MP contamination in the human male reproductive system, highlighting the presence of various MP characteristics in different regions and providing essential data for assessing the risk of MPs to human health. Further investigation should be conducted to determine the potential implications for male reproductive health and fertility.

2.3. Human Placenta

The first evidence of MPs in human placenta was found in a study by Ragusa et al. in 2021. Six human placentas, collected from consenting women with normal pregnancies, were analyzed using Raman microspectroscopy to detect the presence of microplastics.[34]

The placenta regulates the fetal to maternal environment and, indirectly, the external environment. The potential presence of MPs in this organ can have an impact on embryo development, causing risks for the newborn. A total of 12 fragments, ranging from 5 to 10 µm in size and various shapes, were found in four of the placentas. Specifically, five fragments were located on the fetal side, four on the maternal side, and three within the chorioamniotic membranes. The analysis also revealed the presence of industrial pigments like iron hydroxide oxide and ultramarine blue, both applied for cosmetic formulations, such as BB creams, foundations, lipstick, mascara and eyeshadow.

Differently, Braun et al. used FTIR to analyze particles larger than 50 µm and discovered various MPs, including PE, PP, and polyurethane (PU) in two out of three placentas contained.[35]

More recently, three additional studies have been conducted on placentas using different techniques.

In another work, Ragusa et al. examined 10 human placentas using variable pressure scanning electron microscopy (PV-SEM) and transmission electron microscopy.[36] They detected MPs, ranging in size from 2.1 to 18.5 µm, in both intra- and extracellular compartments of different placental cellular layers, including lysosomes, peroxisomes, lipid droplets, multivesicular bodies (intracellular), stroma, endothelial cells, and pericytes (extracellular). Zhu et al. and Liu et al. used laser direct infrared spectroscopy (LD-IR) to investigate MPs in human placentas. Zhu et al.[37] identified PVC (43.27 %) and PP (14.55 as the main polymer types (out of 11 types) found in the placenta. These microparticles ranged in diameter from 20.34 to 307.29 µm, with the majority (80.29 %) being smaller than 100 µm. Liu et al. predominantly found polyamide and PU, which accounted for over 78 % of the MPs.[38] Compared to previous studies, the laser direct infrared spectroscopy (LD-IR) technique detected a higher number of microplastics, suggesting that placentas may accumulate more microplastics than previously estimated. Therefore, it is crucial to gain a thorough understanding of the sources of microplastics in the human placenta, along with their potential health impacts on fetal development.

2.4. Respiratory and Gastrointestinal Systems

Several studies have focused on MP exposure in the respiratory and gastrointestinal systems, which are the primary entry routes for MPs.

In 2021, Amato-Laurenço et al. detected MPs in 13 out of 20 human pulmonary tissue samples obtained during autopsies.[39] These samples were collected from the distal and proximal regions of the left lung of non-smoking adults. Using µRaman spectroscopy, the most frequently determined polymers were PE (24.3 %) and PP (35.1 %). All particles were smaller than 5 µm, while fibers ranged between 8.1 and 16.8 µm.

In 2022, Huang et al.[40] conducted a study where they examined human sputum samples collected from 22 patients with various respiratory conditions to investigate inadvertent inhalation of MPs. FTIR microscopy and laser infrared imaging spectrometry were used to detect MPs in the respiratory tract. The study identified 21 different types of MPs in the sputum samples, with PU being the most common type, followed by polyester (PES), chlorinated polyethylene (CPE), and alkyd varnish, which together constituted 78.36 % of all MPs detected. The study concluded that MPs are prevalent in sputum samples, indicating that inhalation could be a significant pathway for plastics to enter the human body. Moreover, statistical analysis ($p < 0.05$) indicated that the levels of specific MP types found in the respiratory tract were associated with factors such as smoking and invasive medical procedures.

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2.5. Kidneys and Urine

Pironti et al.[41] examined urine samples from six volunteers residing in different cities in Southern Italy. The researchers utilized μRaman to analyze the samples and identify MPs. The results of the analysis revealed the presence of four pigmented microplastic fragments, ranging in size from 4 to 15 μm, with irregular shapes. Specifically, polyethylene vinyl acetate, PVC, PP, and PE were the polymers identified in the samples.

Massardo et al. investigated the presence of microplastics in human kidneys and urine using microRaman spectroscopy.[42] Healthy sections from ten nephrectomized kidneys and ten urine samples from healthy donors were analyzed using micro-Raman spectroscopy.

A total of 26 out of 66 microparticles were identified in both kidney and urine samples, with sizes ranging from 3 to 13 μm in urine and from 1 to 29 μm in kidneys. The most frequently detected polymers were polyethylene and polystyrene, while hematite and Cu-phthalocyanine were the most common pigments. This preclinical study demonstrates the presence of microplastics in renal tissues and confirms their presence in urine, providing evidence of microplastic deposition in human kidneys.

Based on these findings, there is a need for consolidation of actions and further research is necessary to enhance our understanding of the potential toxicity of MPs in humans.

3. Current Global Regulation on Microplastics in Cosmetic Formulations

As stated, microplastics are an emerging environmental and human health problem. Due to their small size, filtering them out in wastewater treatments is challenging, allowing easy access of microplastics to the sea.[16] As a result, for decades, governments and non-governmental organizations (NGOs) have reported the problem of marine pollution caused by plastics and microplastics (Figure 3).

The 2000s marked the beginning of international efforts to address marine litter and protect the aquatic environment. In 1972, the London Convention[43] listed materials considered marine waste including plastic or other synthetic substances. Both the Oslo Convention (1974)[44] and the MARPOL (Annex V-1978)[45] of the International Maritime Organization (IMO) forbade the dumping of materials such as plastics and garbage plastic bags in the sea. On November 3rd 1995,[46] 108 governments, the European Commission with various UN bodies and UN specialised agencies declared the "Global Programme of Action of the Marine Environment" to establish a global initiative on marine pollution in 2003. It stated that domestic wastewaters were discharged improperly. It was noted that 80% of plastic pollution originated from land, and uncontrolled combustion of plastic could generate Persistent Organic Pollutants (POPs), metals, and hydrocarbons. In 2016, UNEA (United Nations Environment Assembly) recognized the negative impacts of microplastics and encouraged countries to take

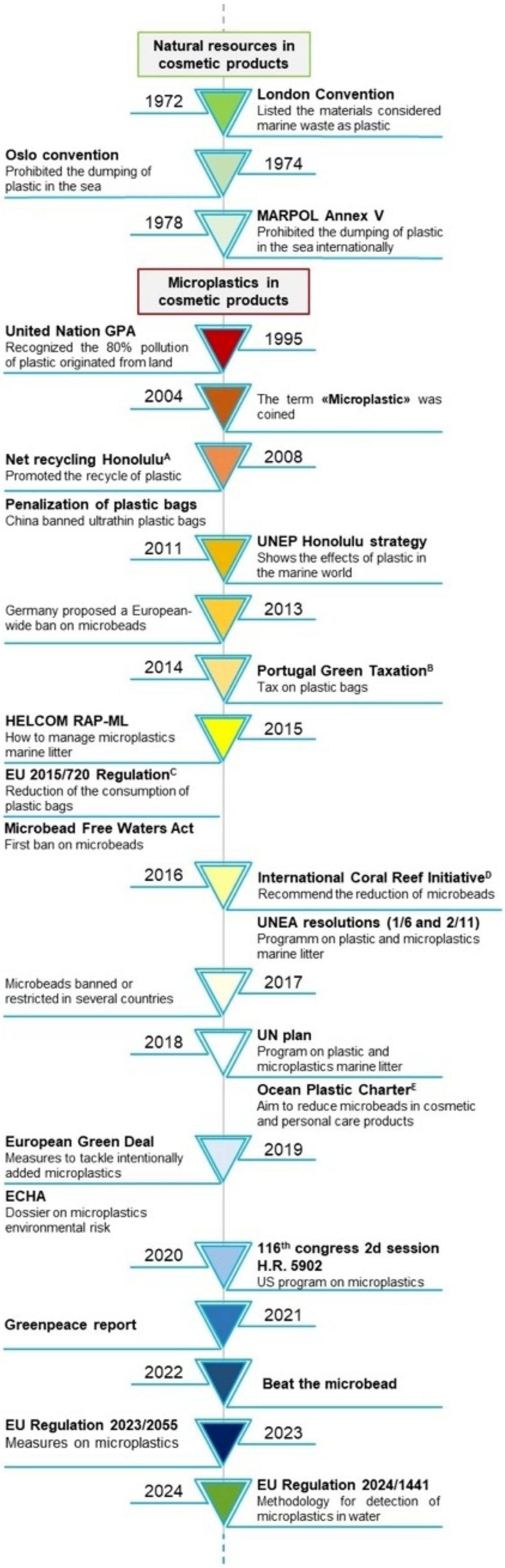

Figure 3. Main actions taken by governments, institutions and associations against microplastics since their introduction.

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immediate actions.[47] In the following years, several documents were published such as the UN plan (2018), and European Green Deal (2019) which suggest solutions to reduce MPs.[48,49] In 2021, Greenpeace published a report where 664 cosmetic products were analyzed and found that 25% of them contained MPs, providing a list of toxic substances along with their relative percentages in each product.[11,50] Other NGOs are monitoring and fighting for marine litter such as "the Honolulu Strategy".[51] In 2019, the European Chemical Agency (ECHA) proposed the definition of microplastic as a "solid polymer-containing particle" and suggested their restrictions.[52,53] Countries have begun to establish new norms and prohibitions on the use of microplastics in commercial products, especially cosmetics(.

3.1. Europe

In 2013, the Dutch government was the first country to propose a European-wide ban on microbeads. A year later, the European Commission established the prohibition of the Ecolabel sign on rinse-off cosmetics.[54] Therefore, Austria, Belgium, Luxembourg and Sweden demanded a ban on microbeads in personal care products in a common statement. A few countries, including the Netherlands and the UK, declared themselves free from microbeads by 2017, while others proposed temporary bans, like Denmark. Italy decided to reduce the use of microplastics in rinse-off products (as well as cotton buds and detergent). From 2020, the marketing of rinse-off manufactures with microplastics are forbidden.[55] The Nordic Council, in January 2017, proposed a full ban on microplastic in cosmetic products.[56]

Two years later, Ireland was the first country to publish the definition of microplastic to form a national ban and in 2020, to eliminate in household and industrial cleaner microbeads.[57] Moreover, European personal care industries started reducing the use of microbeads by 82% by 2015 with Cosmetic Europe introducing further restrictions in 2017.

Furthermore, the European Commission accelerated the implementation of the "Circular Economy Action Plan" which was initially outlined in 2015.[58,59] HELCOM (Helsinki Commission) described how to manage marine litter. It defined "primary" and "secondary" microplastics and how to tackle this issue.[60] On November 9th 2017, the European Commission invited the European Chemical Agency (ECHA) to design a dossier for the restrictions on synthetic water-insoluble microparticle polymers of 5 mm. ECHA published the dossier highlighting the environmental risk of marketing synthetic solid microparticles. Specifically, it proposed a "prohibition of the placing on the market of any solid polymer contained in microparticles or microparticles which have a solid polymer surface coating, as a substance on their own or in a mixture in a concentration equal to or greater than 0,01% by weight".[61] Seven years later the European Union banned products containing added microplastics as cosmetics, personal care and single-use manufactures.[62] Europe aims to reduce the amount of MPs released in the environment by 30% by 2030.[63] Additionally, they established a methodology to measure MPs in water by 2024.[64]

3.2. America

In 2014, Illinois became the first state in the United States to ban non-biodegradable microbeads in personal care products.[65] In 2020, the use of daily chemical products containing microbeads smaller than 5 mm, such as rinse-off products, was banned. By December 2022, the sale of these products was also prohibited.[66] Later, California expanded this ban to biodegradable microplastics and in 2015 microbeads were banned in cosmetics and personal care products.[67,68] In the same year, the US government published the "Microbeads free-water Act" which prohibits the manufacture and sale of microbeads in rinse-off personal care items from 2018.[69] In 2015, Canada labelled microplastic as a toxic substance in the Canadian Environmental Act of 1999, but it was not simultaneously banned. Two years later, regulatory measures banned the manufacture, import, and sale of microbeads in toiletries by July 1, 2018.[70] In 2020, it proposed a system that reduce the use of microbeads in personal care products.[71] In 2016, the Brazilian government banned the use of non-biodegradable plastic in cleaning, bleaching, grinding, and exfoliating cosmetics. Four years later Argentina banned the production, import, and marketing of microbeads in toiletries and makeup items.

3.3. Asia and Oceania

In 2008, the Chinese government penalised plastic bags and launched microplastic monitoring in 2016.[72,73] It proposed guidance for industries to forbid the manufacture of household chemical products containing plastic microbeads. In 2020, banned daily chemical products with microbeads smaller than 5 mm like rinse-off products. In December 2022, their sale was forbidden. Taiwan and South Korea have already banned microbeads in rinse-off cosmetics, while India is still considering regulations to address them.[74] Since 2020, Thailand has forbidden microbeads in cosmetic products to reduce primary microplastics[75] In 2015, Australian government began to take action on microbeads through the NSW EPA.[76,77] During the Meeting of Environment Ministers in December, an agreement was reached to slowly get rid of microbeads in personal care, cosmetic, and cleaning products. Australia eliminated microbeads in rinse-off cosmetics and personal care products. However, New Zealand took action three years later, in 2018, by choosing to prohibit microbeads.[78] Other countries, like the United Arab Emirates (UAE), have not yet announced legal restrictions or bans on the phase-out of microplastics from cosmetics. The situation in South Africa is similar to India, where the government is still consulting on regulations against the use of microbeads in cosmetics.[79] Anyway, in active bans, there is not a universal document which states what microbeads are restricted and for what purpose. There is only an agreement between countries on the definition and size of microbeads.

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4. Major Microplastics in Cosmetic Formulations

As previously mentioned, cosmetic powders are among the most relevant raw materials for the achievement of final formulations with specific properties distinguishable by the end consumer. The small size, shape, and chemistry of these materials are the characteristics that influence most of their properties. Microplastics have led to a drastic change in the performance of formulations, improving sensory attributes, albeit at the expense of sustainability. Table 2 summarizes the principal microplastics exploited in cosmetic products, showing their chemical formula and main effects in formulations.[7,80]

- Polyethylene (PE) is one of the most widely used synthetic polymers and is easy to produce. PE consists of repetitive units of ethylene groups, which give the polymer flexibility and strength. Indeed, the relative polymerization can yield branched or linear structures, leading to the production of high-density polyethene (HDPE), low-density polyethene (LDPE), and linear low-density polyethene (LLDPE), each contributing to different performances. In cosmetics, PE is a film-forming and viscosity-regulating agent.[7,80,81] Specifically, PE microparticles were used in formulations for facial and body scrubs as an exfoliating agent. But since 2018, they have been banned from cosmetic products due to their impact on marine ecosystems.[80]
- Polypropylene (PP) is a thermoplastic homopolymer produced from 1-propene, a gas derived from petroleum and natural gas. PP is known for its lightweight nature and resistance to corrosion, wear, and moisture. This polymer is used as a viscosity-controlling agent in foundations and nail polishes – also, employed as an exfoliant in some formulations.[80]
- Poly(methyl methacrylate) or PMMA is a transparent thermoplastic polymer obtained from methyl methacrylate. In cosmetics, this polymer is used in nail polish, lip gloss, and mascara due to its film-forming aptitude. PMMA microparticles are also used as absorbent agents for transporting active ingredients and as brightness/mattifying regulators, reducing imperfections caused by ageing. Additionally, they modulate the texture of formulations due to their small size.[7,80,82]
- Polytetrafluoroethylene (PTFE) is a homopolymer derived from the polymerization of tetrafluoroethylene. This polymer is used as a hair conditioning and additive in makeup products,[80] including foundations, face powders, and primers, to offer a matte finish. PTFE microparticles can enhance the sensorial experience of cosmetic formulations by improving the texture with a smooth and silky sensation. Additionally, they exhibit non-stick, free-flowing, moisture-resistant, and long-lasting properties, which well-suite for most products.[83,84]
- Polyethylene Terephthalate (PET) is derived from terephthalic acid and ethylene glycol. PET microparticles can be utilized in various formulations due to their properties.[7,80,85] They can enhance the texture of semi-solid formulations and serve as exfoliating agents in skincare products like face and body scrubs. Additionally, they can function as mattifying agents in creams, lotions, and foundations. These microplastics can be used also to deliver and release active ingredients. In addition, they can be coloured and used as additives in the formulation of leg and body paints, nail polish, lip gloss, and hair colouring products.
- Polyamides (Nylon) are synthetic polymers containing amide bonds as repeating units, and various types can be obtained based on the starting monomers; eminent examples are Nylon-6 and Nylon-12.[7,80] The first one is derived from the ring-opening of caprolactam, serves as a viscosity-controlling, bulking, thinning, and moisturizing agent. Nylon-12, a synthetic polymer derived from ω-amino lauric acid monomers, possesses bulking and opacifying properties and is commonly found in skin creams and face powders.
- Polyurethane (PU) is a synthetic polymer made from the reaction between polyols and diisocyanates. In cosmetics, PU microbeads are used as exfoliating, texture enhancement, and/or binding agents. They are capable of absorbing excess sebum from the skin, making them useful in oil-control formulations. Polyurethane can also be used as a colour additive to enhance the effects in products such as nail polish, lip gloss, and eyeshadows.[86]
- An example is PU cross polymer-1, a synthetic copolymer made of isophthalic acid, adipic acid, hexylene glycol, neopentyl glycol, dimethylolpropanoic acid, and isophorone diisocyanate units, which is used as a binding, film-forming, and hair-fixing agent.[80]
- Polyacrylates are a family of synthetic polymers made up of acrylate monomers, which are used as film-forming, antistatic, binding, hair fixative, and suspending agents.[7,82] There are various acrylate copolymers such as styrene-acrylates copolymers, used as colour opacification and filming agents; and ethylene/acrylate copolymers, used as a binding, emulsion stabilizing, thickening, film-forming, and opacifying agent.
- Polyethylene glycols (PEGs) and polypropylene glycols (PPGs), synthetic polymers of ethylene glycol broadly used also in pharmaceutical formulations for drug delivery. They are employed in cosmetic products as humectants or emulsifiers formulated as creams, lotions, and serums. PEGs and PPGs are soluble in aqueous and organic phases, favoring the solubilization of active ingredients and fragrances in formulations.[87]
- Polymethylsilsesquioxane is a synthetic silicone obtained from the hydrolysis and condensation of silicone methyltrimethoxysilane. This synthetic polymer is used for film-forming, texturing, opacifying, skin and hair conditioning.[80,88,89]
- Other microplastics of common usage[7,80] are polybutylene terephthalate (PBT), polystyrene (PS), various types of Nylon, acrylates[82,86,90] and polyurethane crosspolymers.[90]

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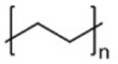
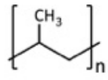
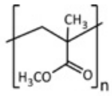
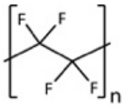
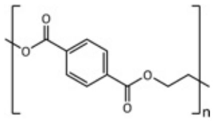
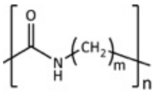
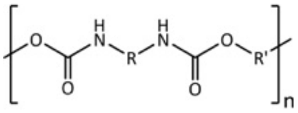
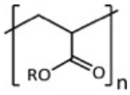
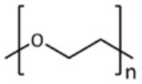
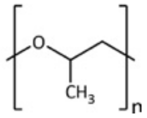
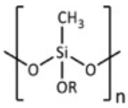
Table 2. Main formulation effects of principal microplastic in cosmetic products.

Microplastic	Formulation effects
 Polyethylene (PE) Polyethylene (PE)	Abrasive, film-forming and viscosity-regulating
 Polypropylene (PP) Polypropylene (PP)	Exfoliating and viscosity-regulating
 Polymethyl methacrylate (PMMA) Polymethyl methacrylate (PMMA)	Brightness/mattifying regulators, carrier of actives, film-forming and texturizing
 Polytetrafluoroethylene (PTFE) Polytetrafluoroethylene (PTFE)	Binding, bulking, hair and skin conditioning, long-lasting, moisture-resistance and texturizing
 Polyethylene Terephthalate (PET)	Absorbent, film-forming, mattifying and texturizing
 Polyamides (Nylon) Polyamides (Nylon)	Bulking, conditioning, opacifying, texturizing and viscosity controlling
 Polyurethane (PU)	Binding agent, film-forming, hair fixing and conditioning and viscosity controlling
 Polyacrylates Polyacrylates	Absorbent, binding agent, emollient, emulsifying filming, hair and skin conditioning, opacifying and viscosity controlling
  Polyethylene glycols (PEGs) Polypropylene glycols (PPGs)	Emulsifying, hair and skin conditioning, humectant and solvent
 Polymethylsilsesquioxane Polymethylsilsesquioxane	Film-forming, hair and skin conditioning, opacifying and texturing

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5. Eco-friendly Cosmetic Powders – Alternatives to Microplastic

The regulatory restrictions applied to plastic microbeads stem from growing scientific evidence of their environmental release and harm. However, the presence of alternative materials has played a key role in driving the implementation of these regulations.[91] Numerous initiatives are underway to address the harmful impacts of microplastics, including those originating from personal care and cosmetic products, on marine environments and other ecosystems. Leading these efforts is the replacement of traditional synthetic microplastics with eco-friendly, sustainable, and biodegradable materials.[79] In the cosmetic industry, there is active seeking of alternatives to traditional microplastic ingredients, distinguishing natural, semi-synthetic and synthetic alternatives as Figure 4 shows.[92]

5.1. Natural Alternatives

Consumer consciousness about the detrimental impact of synthetic polymers on the environment is pushing for the improvement of biopolymer production from natural sources. The eco-friendly, safe, and biocompatible traits of natural polymers make them particularly significant in cosmetic formulations. Natural polymers like starch, cellulose, alginate, chitosan, lignin, pectin, xanthan gum, agar, hyaluronic acid, guar gum, gelatine, collagen, and keratin can be modulated as microparticles[93–95] and formulated for the cosmetic industry.[96] These versatile materials can be employed in skincare, haircare, and makeup, serving as stabilizers and modifiers. Their safety, biocompatibility, eco-friendliness, and appeal to consumers make them highly suitable for various cosmetic applications. Introducing small molecules or polymers can modify natural alternatives, resulting in semi-synthetic materials. Celluloses and starches are commonly modified to gain properties for skin sensoriality and formulation purposes.

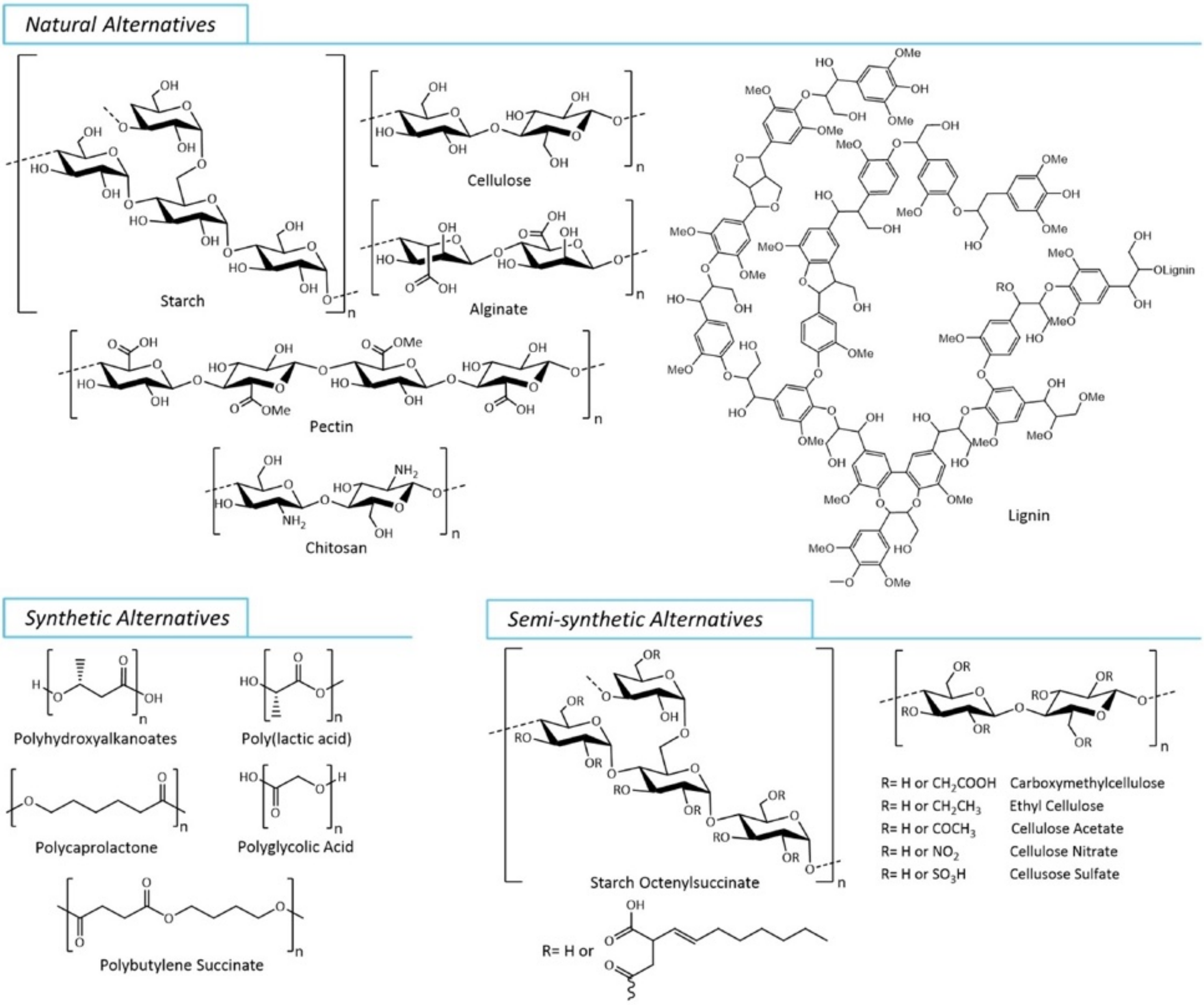

Figure 4. Main natural, semi-synthetic and synthetic alternatives used to replace microplastics in cosmetic products.

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5.2. Starch

Starch is a natural polysaccharide obtained from plant sources such as corn, rice, maize, wheat, and barley. This biopolymer usually exhibits size distribution in the order of micrometers.[97] It is utilized as a versatile cosmetical component, applied in skincare, hair care, and personal care formulations. Starch beads have garnered significant attention in cosmetic formulations due to their biodegradability, renewability, and potential to replace non-biodegradable microplastics in personal care products.[96,98]

A study by Junlapong et al. investigated the biodegradation of starch-based hydrogels resulting in environmentally friendly alternatives to synthetic microplastics.[99] This feature aligns with the growing consumer demand for sustainable and eco-friendly cosmetic products. Studies have also shown that controlling parameters such as starch source, processing techniques, and crosslinking agents can influence the size, shape, and texture of starch beads, thereby affecting their suitability for various cosmetic formulations.[100] For example, Farrag et al. pointed out that the source of starch significantly influences the self-assembling behavior of this polymer at the nanometric level due to different amylose/amylopectin ratios. Starch beads have been explored as carriers for active ingredients in cosmetics, improving stability, controlled release, efficacy, and longevity.[101] For instance, Adejoro et al. succeeded in producing starch-based systems enabling the production of homogeneous microcapsules trapping tannins.[102] Pueknang and Saewan encapsulated folic acid in phosphorylated rice starch producing a semi-solid formulation. They evaluated the improvement of human skin noticing the melanin content, scaliness, and wrinkle.[103] Starch beads can also serve as texture enhancers in cosmetic formulations by providing desirable sensory properties such as smoothness, spreadability, and a luxurious feel. Studies have investigated the rheological properties of starch-based gels and emulsions, demonstrating their potential to improve the texture and consistency of creams, lotions, and other cosmetic products.[104] Research by Marto et al. has focused on assessing the compatibility and stability of starch-based emulsions (St-BV) in various cosmetic formulations. The physicochemical characteristics and the toxicological profile of ingredients combined with the risk characterization and the evaluation of tissue viability resulted in being safe for human use. Furthermore, an increase in skin hydration and microcirculation has been observed.[105]

Besides the above-mentioned advantages, starch is one of the easily modifiable polymers due to its simple structure and abundance in nature. Different physical properties could be achieved by to the polymer through the grafting of molecules and/or macromolecules, making the resulting starch derivatives one of the most versatile alternatives in the formulation field.[106] A common modification involves the insertion of octenyl succinic acid (OSA) to confer hydrophobic character to starches, leading to a smart product primarily employed as an emulsifier. Mu et al. modified waxy maize and corn starches with 3 % OSA to create oil-in-water emulsions and studied their stability under various conditions (pH change, electrolyte concentration, and enzymatic treatment). OS-starches can be customized for the release of active ingredients by controlling their destabilization rate.[107]

5.3. Cellulose

Cellulose is a natural polymer found in the cell walls of plants and is derived from abundant sources, including wood, hemp, cotton, and linen. Macro- and nanofibers originated from cellulose are a highly appealing substrate for cosmetics, as they are biodegradable, non-toxic,[108] and offer gentle exfoliation without causing harm to the environment. Celluloses are mainly employed as emulsifiers, film-forming and thickeners agents in cosmetic formulation, i.e. facial scrubs and exfoliating cleansers.[96,109]

OBrien et al. produced spherical cellulose microbeads by a scalable membrane emulsification–phase inversion process as an eco-friendly alternative to microplastics.[110] Recently, bacterial cellulose (BC) is taking place in this field due to its purity, porosity tensile strength. BC has identical chemical composition with pure plant-derived cellulose, differing solely in molecular weight.[111] This polymer can be used in cosmetic products as Personal Care formulations, facial scrubs and mask.[112,113]

As already mentioned, cellulose is often modified to achieve characteristics useful for formulating cosmetic products, i.e. improved solubility – some examples are sodium carboxymethylcellulose, ethyl cellulose, and cellulose esters.

Sodium carboxymethylcellulose (NaCMC) is the sodium salt of carboxymethylcellulose (CMC), resulting from the etherification of cellulose with sodium monochloroacetate in an alkaline solution (NaOH). NaCMC is a water-soluble biopolymer used in cosmetics as a moisturizer, humectant, and emulsifier. In a study by Martins and Rocha, CMC was tested with bacterial cellulose, as an emulsifier in a cosmetic cream. This combination was able to completely replace commercial surfactants maintaining the rheological properties of the formulations.[114] Aguiar et al. produced spherical Na-CMC microparticles by spray drying to encapsulate three natural antioxidants – caffeic acid (CAF), chlorogenic acid (CGA) and rosmarinic acid (RA). They obtained high encapsulation efficiency and evaluated the total release and the antioxidant activity.[115] Instead, Costa et al. used CMC as a bioactive cosmetic ingredient for skincare formulation. They studied the effects in HaCat and HDFa cells showing no cytotoxic effect, intracellular production of procollagen I α I and modulation HaCat immune response.[116]

Ethylcellulose (EC) is a non-ionic water-insoluble cellulose ether obtained from the etherification of alkali cellulose with ethyl chloride.[117] Juleaha et al. encapsulated essential oils isolated from the peel of *C. aurantifolia* in ethyl cellulose microparticles by coacervation method for cosmetotextile products.[118]

Cellulose esters are obtained from the reaction of natural cellulose with organic acids, anhydrides, and acid chlorides. Generally, they are water-insoluble polymers, characterized by excellent film-forming properties.[119] These polymers are mostly used as gelling agents, bioadhesive, thickening, and stabilizing

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agents applied in cosmetic formulations (creams, shampoos, and lotions).[92] Some examples of cellulose esters are cellulose acetate (CA), cellulose sulfate (CS), and cellulose nitrate (CN).

5.4. Alginate

Alginate is an indigestible polysaccharide naturally produced and typically harvested from brown algae. The molecular structure consists of unbranched linear binary copolymers comprising β-D-mannuronic acid (M) and α-L-glucuronic acid (G) residues linked by 1,4-glycosidic bonds. In algal alginate structures, three uronic acid blocks are present, including homopolymeric regions of M and G blocks, as well as alternating MG blocks containing both polyuronic acids. Typically, bacterial alginates contain O-acetyl groups absent in algal alginates, with bacterial polymers exhibiting higher molecular weights.[120] The ability of sodium alginate (SA) to create a physical hydrogel through ionic crosslinks, particularly with divalent cations like Ca^{2+}, is well-documented. These alginate hydrogels undergo biodegradation through the hydrolysis of glycosidic linkages and are also subject to degradation due to the release of divalent cations.[121] In their study, Bae et al. produced sodium alginate (SA) microbeads (MB) by using an aqueous solution of SA and electrospraying it into a Calcium water solution. An increase in microbead size from 640 to 880 μm was observed by the SA concentration and the nozzle diameter, showing how these parameters can influence the final dimension and shape of the product.[122] Given their mucous consistency, cost-effectiveness, non-toxic nature, and biocompatibility, alginate hydrogels find extensive use in various biomedical and environmental applications such as drug delivery systems, cell encapsulation, and cosmetics.

5.5. Chitosan

Chitosan is a biopolymer derived from deacetylation of chitin, a natural polysaccharide found in the exoskeletons of crustaceans like shrimp, crab, and lobster, as well as in the cell walls of fungi.[123] Chitosan can be used in cosmetic formulations for skin and hair care products. Specifically, it is utilized as a texturizer, emulsifier, film-forming and humectant agent.[124,125] Gomaa et al. utilized chitosan microparticles as carriers of Ensulinzol (PBSA) to apply sunscreen products.[126] Ju et al. prepared chitobeads (CBs) using chitin, which, after re-acetylation, exhibited higher cleansing efficiency compared to polyethylene beads able to remove potentially toxic elements.[127] Instead, Wisuitiprot et al. tested a cream containing chitosan microparticles loaded green tea extract on human facial skin, demonstrating anti-wrinkle effects and improved skin elasticity and lightening after 2 months treatment period.[128]

5.6. Lignin

Lignin is a polyphenolic material present as main component in the plant cell walls and obtained by oxidative coupling of three monolignols, para-coumaryl alcohol (H), coniferyl alcohol (G) and synapyl alcohol (S). Depending on the degree of these monomers (H, G and S), the lignin can be classified in Grass Lignin, Softwood and Hardwood. In general, lignin presents several properties useful for topic applications in cosmetics. In fact, it is an emulsion stabilizer, antimicrobial and UV shield agent.[129] Antunes et al. exploited lignin from sugarcane bagasse in a semisolid formulation demonstrating its activity to scavenge ABTS and DPPH radicals. Moreover, their study revealed an in vivo Sun Protection Factor (SPF) value of 9.6 ± 0.8, indicating a broad-spectrum UV protection capability.[130] Lee et al. employed lignin under mild conditions (MWL) as sunscreen agent, demonstrating synergistic effects with commercial ones, and enhancing SPF activity of commercial product.[131]

5.7. Pectin

Pectin is a natural polysaccharide present in the cell walls of fruits and vegetables, such as apples and berries and is made of a network of galacturonic acid with side chains of other sugars, such as rhamnose, arabinose, and galactose.[132] This conformation provides specific properties making pectin employing as a gelling agent, thickener, and stabilizer.[95]

In literature, there are few techniques to produce pectin microparticles (i.e., ionic gelation, spray drying, and extrusion), especially for encapsulating active ingredients, but mostly related to the pharmacological field as drug delivery systems.[95,132,133]

5.8. Synthetic Alternatives

Synthetic polymers deriving from renewable sources such as polyhydroxyalkanoates and poly(lactic acid), are gaining attention as an alternative to petrochemical-based plastic due to their biocompatibility, biodegradability, and non-toxicity.[96,134]

5.9. Polyhydroxyalkanoates (PHA)

Polyhydroxyalkanoates (PHAs) are polyesters of hydroxyalkanoates (HAs) and naturally produced by various Gram-positive and Gram-negative bacteria. PHAs can be obtained by fermenting renewable sources making them a sustainable alternative to petrochemical-based plastics such as PET. The number of carbon atoms in the chain determines the structure and types of PHAs. PHA is biodegradable and biocompostable, making it an eco-friendly option for a range of products including cosmetics. PHAs are used in many beauty products such as beauty masks or sanitary pads, as well as in heat-sensitive adhesives, smart gels, and surfactants.[135,136] They have antiox-

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idant and moisturizing properties and products containing PHAs can have an antibacterial effect.[96] PHA has tiny pore sizes, high dependability, and high surfactancy. They offer many advantages like easy manufacturing, good UV resistance, and hydrophobicity.[137] Phothong et al. investigated astaxanthin-loaded PHB microbeads, from crude glycerol, for facial scrubs not observing skin irritation and sensitization during a human repeated insult patch test (HRIPT).[138]

5.10. Poly(Lactic Acid) (PLA)

Poly(lactic acid) (PLA) is an aliphatic thermoplastic polyester composed of lactic acid (2-hydroxy propionic acid) units obtainable from renewable and degradable resources like corn and rice. PLA has a low environmental impact because its degradation yields mainly water and carbon dioxide. Poly(lactic acid) is commonly employed in cosmetic packaging due to its mechanical resistance and good rigidity. PLA is ideal for applications on skin, hair, and nails finding use in cosmetic products including makeup, scrub soaps, creams, gels, and lotions.[139]

5.11. Polyglycolic Acid (PGA)

Poly(glycolic acid) (PGA) is a biopolymer synthesized via the condensation of glycolic acid or by the ring-opening of glycolide. PGA is insoluble in a wide range of solvents because of its high porosity, hydrophobicity, and high crystalline structure (between 45% and 55%).[140] PGA has better mechanical qualities and a faster rate of deterioration than polylactic acid (PLA).

5.12. Polycaprolactone (PCL)

Polycaprolactone (PCL) is an aliphatic polyester made of hexanoate units, characterized by its hydrophobic and semi-crystalline structure. PCL is an eco-friendly biopolymer obtainable from renewable materials.[141] For example, Forigua et al. produced PCL microparticles w/o cargo exploiting a microfluidic system. The size distribution below 50 μm and the spherical shape obtained are very promising not only in drug delivery and tissue engineering, but also in cosmetics.[142] Nam and Park produced PLA and PCL MBs using an eco-friendly melt electrospraying process, without the need for any organic solvent. These aliphatic polyester-based biodegradable MBs showed high skin hydration and minimal irritation, making them suitable for use in cosmetics.[143]

5.13. Polybutylene Succinate (PBS)

Polybutylene Succinate (PBS) is a biopolymer derived from the polycondensation between 1,4-butanediol and succinic acid, obtainable from renewable sources such as sugar cane. It degrades in non-toxic products such as water, carbon dioxide, and biomass. PBS is known for strength, stiffness, toughness, biodegradability properties showing resistance to various chemicals, and compatibility with a wide range of additives.[144] Dutra et al. proposed a new production of PBS microparticles through a water-free suspension polycondensation process with an average size of 80–180 μm.[145] Gan et al. synthesized four polyester microparticles, including PBS and poly(butylene succinate adipate) (PBSA), and tested their degradation under marine and enzymatic conditions, showing promising results compared to the common microplastics used in cosmetics.[146]

6. Summary and Perspectives

Although many natural alternatives exist and can be modified using functional groups like amines, carboxylic acids, aldehydes, and thiols, there are currently fewer patents in this area compared to the number of products on the market.This occurs because companies, both for reasons of trade secrecy and for a rapid transition to plastic-free and vegan products, utilize natural microbeads without making modifications. However, products or formulation examples containing microplastics or 'skeptical microplastics' (synthetic polymers lacking enough available information and are under investigation) are still observed, to enhance properties (i.e. film forming, long-lasting effects, and others).

Despite the challenging and rapid transition to sustainable formulations, there has been an increase in patents where both natural and synthetic alternatives are discussed to be employed in the final product, contributing to its main characteristics.

Starches and celluloses are receiving increased attention due to their wide availability from various plant sources globally. Different types of starches like ginger, turmeric, wheat, and mung bean are used to make solid and semi-solid products such as eyeshadow and emulsions.[147] Additionally, starches can be used to create innovative solid formulations for hair products, primarily composed of polyols, fatty alcohols and/or acids, improving the shelf life and avoiding the transport of a liquid composition.[148] In combination with xanthan gum and cellulose, starch is used as a thickening agent to obtain an eco-sustainable stabilizing component for make-up products such as mascara, powder foundation, lipstick, lip gloss, and nail polish, as well as for hair and skin care.[149]

Celluloses also find their application in producing cosmetic products,[150,151] often with other ingredients, as previously cited to create products like sunscreen and waterproof formulations without polyacrylates, carbomers, and polyvinylpyrrolidones[152] Other celluloses, such as sodium carboxymethylcellulose and microcrystalline cellulose, combined with Diutan gum, ensure high stability in personal care formulations.[153] Sustainable alternatives such as lignin,[154,155] alginate,[156,157] and pectin[158,159] are already exploited in patented products for these applications.

Synthetic alternatives are gaining ground in cosmetics, particularly polyhydroxyalkanoates (PHA) and polycaprolactone (PCL). PHAs offer several advantages, including the ability to

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adsorb oils, adjust the viscosity of certain thickening agents, and enhance opacity, and uniformity of pigmentation.[160] PHA microparticles are utilized in anhydrous formulations, free of polyamides and acrylic polymers, such as lipsticks, which require good dispersibility, viscosity, moldability, and mechanical properties.[161]

On the other hand, PCL can create spherical microparticles[162] and is used to encapsulate collagen peptides[163] or vitamin C[164] as dermal fillers, as well as metal oxide particles as opacifiers.

As noted, the challenge of removing microplastics from beauty care products is a current topic in the cosmetic industry worldwide. Institutions work to eliminate all components harmful to the ecosystem and promote a sustainable transition. In cosmetics, this approach represents a significant opportunity for innovation due to green consumers pushing companies to reconsider their formulations, also in response to government bans.

The fact that microplastics have now entered the human food and vascular cycle is a cause for serious global alarm.[165] However, scientific literature remains lacking in terms of alternatives aimed at replacing microplastics, especially concerning the necessary sensory characteristics requested by the cosmetic market.

In this review, we have summarized the main types of microplastics (as shown Figure 5a–c), and their characteristics utilized in current cosmetic products such as make-up, skincare, and hair care. We highlight three categories of alternatives (natural, semi-synthetic, and synthetic) that include the most promising solutions currently under investigation (as shown Figure 5d–h). The newly explored biopolymers applied as microplastic substitutes and their main characteristics have been discussed herein.

Natural resources derived from plants, such as starches, cellulose, and lignin, are emerging as the primary alternatives to reformulating cosmetic products, especially for their broad availability in comparison to other ones. These natural ingredients not only offer an ecological solution to the problem of microplastics but also have the potential to improve the overall performance and sustainability of finished cosmetic products.

The pressing need to adopt more eco-friendly approaches is pushing researchers to focus more on current claims. We anticipate a significant increase in literature on sustainable cosmetics in the next few years, as the interest in sustainable cosmetic products is rapidly increasing. Presumably, studies will primarily focus on new production methodologies, the use of natural and biodegradable ingredients, as well as the studies of the environmental impact of new cosmetic formulations. This growing research interest aims to provide companies and consumers with detailed information to support informed and sustainable choices in cosmetic products.

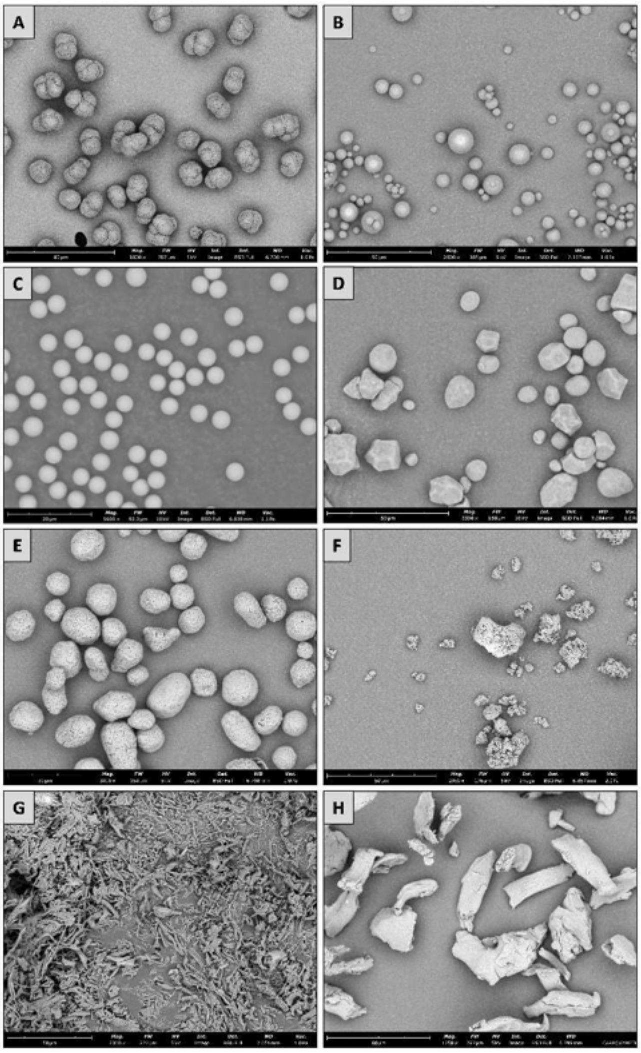

Figure 5. SEM images of A) Nylon 12, B) PMMA, C) polymethylsilsequioxane, D) Corn starch, E) Cellulose beads, F) Lignin, G) PLA, H) Sodium carboxymethylcellulose. Pictures taken in the NanoBioLab using Phenom Pro G6 Desktop SEM.

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Conflict of Interests

The authors declare no conflict of interest.

Keywords: Microplastics · Natural products · Sustainable chemistry · Cosmetic formulations · Regulatory measures

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