Biopolymer-based freeze-dried emulsion and bigel composites with *Sambucus nigra*-loaded chitosan microparticles: novel multifunctional systems for enhanced skin performance

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Abstract: The cosmetic industry is dominated by water-based formulations for skin care and conditioning. Therefore, the novelty of this work lies in reducing water consumption during the production of materials for dermatological and cosmetic purposes, while simultaneously maintaining the functional properties of the prototype based on a freeze-dried emulsion and a bigel with plant extract-loaded microparticles. The formulations were prepared using biopolymers (sodium alginate, whey protein isolate, and ethylcellulose), lipids (sea buckthorn oil and beeswax), cryoprotectant (mannitol), and emulsifier (Span 80). Emulsion and bigel were modified by the addition of chitosan microparticles loaded with *Sambucus nigra* flower extract showing antioxidant properties. The materials were characterized by SEM, mechanical testing, porosity, density, residual moisture content and biophysical skin parameter analysis, including transepidermal water loss (TEWL), skin hydration and color. The results demonstrated that bigels exhibited higher mechanical strength and residual moisture content but lower porosity and density than emulsions. The combination of WPI, sodium alginate, ethylcellulose, mannitol, sea buckthorn oil, beeswax, and elderflower extract provided a synergistic effect, improving *stratum corneum* hydration level and barrier integrity while reducing redness. This research highlights the potential of freeze-dried biopolymer-lipid matrices with embedded microparticles as innovative and eco-friendly cosmetic and dermatological formulations.

Keywords: freeze-drying, emulsion, bigel, microparticles, plant extract

1. Introduction

Microencapsulation is used to obtain spherical particles with diameters varying from 1 to 1000 μm. The most widespread forms of microparticles are microspheres and microcapsules. The substantial difference in their morphology is the distribution of the active substance. Microcapsules exhibit a membrane-wall structure with a core containing the bioactive substance, while microspheres have a matrix system where the bioactive substance is dispersed throughout the particles [1]. Different materials can be used as encapsulating matrices, including biopolymers, copolymers or synthetic polymers, proteins, and lipids [2]. Chitosan is a naturally occurring polysaccharide derived from chitin that provides biocompatible and biodegradable wall material [3,4]. Chitosan's solubility in acidic conditions enables the pH-sensitive release of encapsulated substances, which is especially useful for targeted

delivery, as the skin exhibits a slightly acidic nature [5]. This environmentally friendly polymer, aligning with the growing demand for sustainable biomaterials and degradable polymers [6], forms stable microparticles, providing a protective barrier against environmental factors [7]. The main advantages of particulate systems are the isolation and protection of active substances dispersed in the core from external factors and undesired reactions (e.g. oxidation or deactivation), which simultaneously increases and maintains the stability of these substances. Further reasons for encapsulation are modification of the physicochemical properties, separation of incompatible materials, and masking of organoleptic properties like color, taste and odour of substances [8]. In addition, encapsulation allows the modification of the release of active compounds with three main mechanisms of delivering active substances from particles, namely (i) mechanical rupture of the capsule wall; (ii) dissolution or melting of the wall and (iii) diffusion through the wall [9]. Moreover, the skin's outermost layer, the *stratum corneum*, acts as a strong barrier, limiting the penetration of hydrophilic and large molecular weight compounds. Microencapsulation addresses this challenge by protecting active substances, improving their stability, and ensuring controlled release for gradual diffusion into deeper skin layers. Due to these advantages, microencapsulation plays a crucial role in pharmaceuticals, medicine, cosmetics, material engineering and food sciences, significantly improving the functionality and effectiveness of bioactive substances across diverse applications.

Various active ingredients may be encapsulated into microparticles, including plant extract such as extract prepared from *Sambucus nigra*, widely known as elder. Elderflower and their preparations have already been used for generations in folk medicine in many countries due to their strong beneficial effect on the skin. Besides the healing effect on skin disorders, as well as soothing and regenerating the skin, elderflowers exhibit a wide range of biological activities, such as antioxidation, antibacterial, antiparasitic, neuroprotective, anti-inflammatory, anticancer, antiviral, and antidiabetic properties [10]. This is due to its complex composition since it contains a whole range of phenolic compounds, such as flavonoids (e.g. rutin, quercetin, isoquercetin, isorhamnetin, kaempferol) and phenolic acids (e.g. chlorogenic and caffeic acids). Hence, it has anti-inflammatory properties and stimulates skin renewal [11,12]. Elderflower extract also comprises organic acids (e.g. valeric and ferulic) and smaller amounts of tannins and essential oil.

Incorporating microparticles into freeze-dried materials has gained significant attention in recent years as a strategy to improve the efficiency of active substance delivery and the protection of active substances [13–15]. Porous materials, characterized by their high surface area and tunable pore structures, are suitable carriers for controlled release applications [16]. By embedding microparticles within these matrices, it is possible to enhance active compounds' stability, bioavailability, and targeted delivery, thereby optimizing their therapeutic or functional effects. Freeze-drying of polymeric materials has already been extensively studied [17]. However, freeze-drying of emulsions and bigels presents a novel approach. Emulsions are systems where two immiscible liquids, such as oil and water comprising dispersed and continuous phases, are stabilized by an emulsifier. Bigels are more complex, gel-like semisolid systems formed by combining, through physical interactions, a hydrogel (based on hydrophilic polymers) and an oleogel (oil gelled with an organogelator).

The functionality of these novel formulations is strongly influenced by the polymers used as structuring agents. Natural and semi-synthetic polymers, such as sodium alginate, whey protein isolate (WPI), and ethylcellulose, play a crucial role in defining the physicochemical and mechanical properties of emulsions and bigels. Their gelling, emulsifying, and stabilizing capacities allow the preparation of freeze-dried systems with tunable porosity, mechanical properties, and degradation profiles. Such properties combined with biocompatibility are essential for ensuring both product performance and

controlled delivery of active substances in cosmetic and dermatological applications. WPI, a purified protein derived from milk whey, is rich in essential amino acids and exhibits gelation, film-forming and stabilizing properties [18]. It helps form stable networks, enhancing the mechanical strength and water-binding properties essential for maintaining structural integrity in freeze-dried systems [19]. Sodium alginate, a polysaccharide extracted from brown seaweed, serves as a gelling, thickening and stabilizing agent [20]. It has also been recognized for contributing to the structural integrity of materials by forming a flexible, stable network in aqueous environments. However, ethylcellulose (EC), a cellulose derivative, can be used as a polymeric organogelator due to its ability to directly structure liquid oil properties. EC is also valued for its thermal stability and ability to form transparent gels, making it suitable for various applications, including drug delivery and food formulations [21]. Despite the differences in composition and structure of emulsions and bigels, these two systems are recognized for their advantages. Freeze-dried emulsions and bigels offer enhanced stability and extended shelf life by removing water, preventing microbial growth and degradation of active ingredients. Additionally, they provide controlled rehydration, allowing for efficient delivery of active substances upon restoration, making them ideal for applications in pharmaceuticals and cosmetics.

To the best of our knowledge, there are no reports in the literature (other than our preliminary studies [22,23]) regarding the preparation of materials for cosmetic applications with reduced water consumption and incorporated microparticles containing enclosed active substances. Such materials are needed as they offer a sustainable approach to cosmetic formulation by significantly reducing water consumption and addressing environmental concerns in the beauty industry. Additionally, they enhance the stability and bioavailability of active compounds, ensuring prolonged efficacy and deeper skin penetration.

This research studies the possibilities of using a new class of three-dimensional materials as an alternative cosmetic form for traditional emulsions. The idea of preparing materials that require reduced water usage for preparation (water sublimed during freeze-drying can be reused in the next step of production, decreasing overall water usage) and application (dissolving in a minimal amount of aqueous solvent back to emulsion or bigel immediately before its application to the skin) is an innovation in the field of cosmetic chemistry. Polymeric microparticles with biologically active compounds from *Sambucus nigra* – a plant chosen due to its high content of antioxidant compounds and showing substantial effects on the skin – are incorporated into materials to protect these compounds during the freeze-drying process and storage, as well as their more effective penetration into the deeper layers of the skin. This combines the advantages of both encapsulation and freeze-dried emulsions and bigels.

We hypothesize that water consumption during obtaining cosmetic emulsion can be reduced, simultaneously maintaining the functional properties of the prototype based on freeze-dried emulsion and bigel with plant extract-loaded microparticles. Therefore, the main goal of this research was to modify freeze-dried emulsion and bigel based on biopolymers: sodium alginate, WPI, ethylcellulose; cryoprotectant: mannitol; lipids: sea buckthorn oil, beeswax; emulsifier: Span-80 with the addition of chitosan microparticles loaded with *Sambucus nigra* flower extract. Prepared materials were characterized via SEM, mechanical properties, residual moisture content, porosity, density, and analysis of biophysical skin parameters, including skin color, hydration and barrier quality (transepidermal water loss – TEWL). Instrumental skin analysis is crucial for evaluating topically applied formulations' efficacy and physiological impact, ensuring optimal permeability of active substances while minimazing adverse effects. These materials may become the basis for developing a multifunctional new form of cosmetic and dermatological preparations for skin conditioning and care, increasing the effectiveness of the treatment of many skin conditions.

2. Materials and Methods

2.1. Materials

Sodium alginate (ALG) was obtained from BÜCHI Labortechnik AG (Flawil, Switzerland). Whey protein isolate (WPI) (BiPRO) containing 97.7% protein and 75% β-lactoglobulin in DM was purchased from Davisco Foods International Inc. (Eden Prairie, MN, USA). Ethylcellulose (EC), Span 80, beeswax, and chitosan (ultra-low molecular weight, MW: 20,000 (avg.)) were obtained from Sigma-Aldrich (Poznan, Poland). D-Mannitol and tripolyphosphate (TPP) were obtained from Pol-Aura (Poznan, Poland). Isopropanol was acquired from Stanlab (Lublin, Poland). Sea buckthorn oil and dry plant raw material (flowers of *Sambucus nigra*) were acquired from the herbal wholesaler Nanga (Zlotow, Poland). All chemicals mentioned here were of analytical grade.

2.2. Preparation Method of Plant Extract-Loaded Microparticles

The *Sambucus nigra* flowers extract was obtained using the Soxhlet apparatus with 10 g of dried plant raw material and 200 ml of water during 3 hours of extraction (Fig. 1). Fabricated extract was previously characterized by Total Polyphenol Content – TPC (267.71 ± 6.60 mg/ml), Total Flavonoid Content – TFC (34.36 ± 0.86 mg/ml), and antioxidant capacity based on CUPric Reducing Antioxidant Capacity – CUPRAC (611.24 ± 23.29 mg/ml), Ferric-Reducing Antioxidant Power – FRAP (610.03 ± 26.19 mg/ml) and 2,2-diphenyl-1-picrylhydrazyl – DPPH RSA (78.1 ± 0.5%).

An encapsulator (B-395 Pro, BÜCHI Labortechnik AG, Flawil, Switzerland) was applied using the extrusion method to prepare extract-loaded chitosan microparticles (MPs). A solution of 2% (v/v) acetic acid and elderflower extract in a 50:50 ratio was used to dissolve 2% (w/w) of chitosan. The prepared mixture was transferred into the encapsulator's pressure bottle and forced through a 450 μm diameter nozzle. Droplets separated by an electrical field were cross-linked in an 8% (w/w) tripolyphosphate (TPP) solution. Obtained microparticles were collected and rinsed with distilled water. It was established that obtained chitosan microparticles had 71.4 ± 6.3% loading capacity.

2.3. Preparation Method of Materials

Freeze-drying of O/W emulsions (E) and bigels (B) was the basis of materials preparation [23]. Both material types were fabricated using aqueous and oily phases. The preparation methodology is presented in Figure 1.

The aqueous phase consisted of 3% (w/w) of WPI, 2% (w/w) of sodium alginate (ALG), and 1% (w/w) of mannitol. The hydrogel was heated to 70–80°C on the magnetic stirrer. Whereas the emulsion oily phase comprised sea buckthorn oil, 3% (w/w) of beeswax and 1% (w/w) of Span 80 and, subsequently, it was heated to 70–80°. In order to prepare bigel, 10% (w/w) of ethylcellulose (EC) was added to form oleogel from the oily phase and heated to 140°C. Emulsion and bigel were obtained with a 10/90 oily-to-aqueous mixing ratio. Concentrations were calculated based on the total mass of the formulation. Aqueous and oily phases were mixed and homogenized for 3 min at 20,000 rpm (T25 digital ULTRA-TURRAX disperser, IKA Werke, Staufen, Germany). Emulsion and bigel were cast on glass plates, frozen (−20°C) and freeze-dried (−55°C, 5 Pa, 24 h) (ALPHA 1−2 LD plus lyophilizator, Martin Christ, Osterode am Harz, Germany).

Emulsion (E+MPs) and bigel (B+MPs) were also modified with the addition of chitosan microparticles loaded with elderflower extract. Composite materials were fabricated according to the abovementioned

procedure; however, microparticles at 5% (w/w) concentration based on the total mass of emulsion or bigel were added before casting the solution on the glass plates. The 5% concentration was selected as an optimal level to ensure a balance between functional activity and structural integrity of this materials based on preliminary studies and literature data [22,24–26].

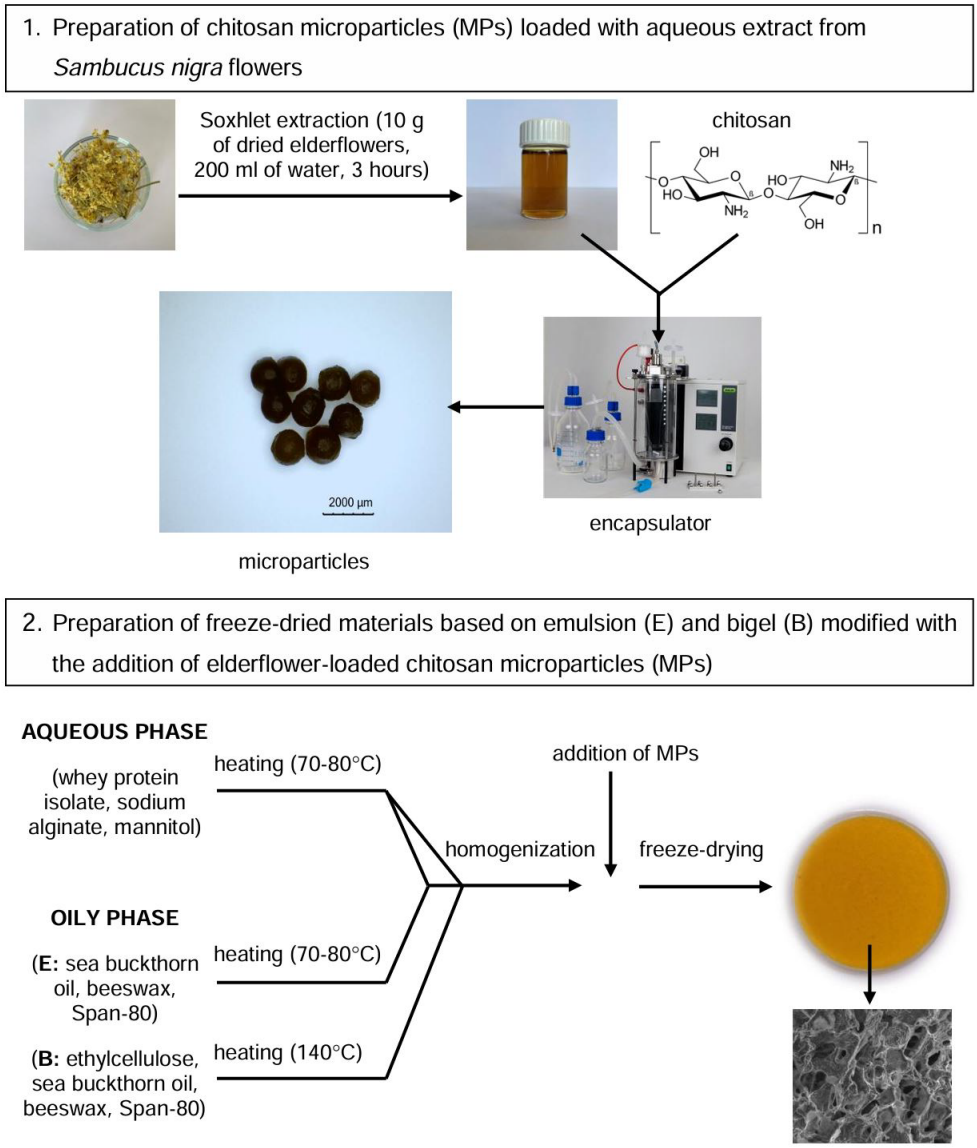


Figure 1. The method of preparation of chitosan microparticles loaded with *Sambucus nigra* flower extract (MPs) and materials based on freeze-dried emulsion (E) and bigel (B).

2.4. Characterization of Materials

2.4.1. Structure of Materials

Scanning electron microscopy (SEM) imaging (Quanta 3D FEG scanning electron microscope, Quorum Technologies, Lewes, UK) was used to analyze the structures and cross-sections of the prepared freeze-dried emulsions and bigels. Prior to the analysis, a thin layer of gold and palladium was applied to the materials' surface using the SC7620 Mini Sputter Coater/Glow Discharge System (Quorum Technologies, Lewels, UK)

2.4.2. Mechanical Properties

Compression of freeze-dried emulsions and bigels was carried out with a mechanical testing machine (Shimadzu EZ-Test EZ-SX, Kyoto, Japan) fitted with a 50 N load cell. Materials were cut into seven cylindrical samples with 10 mm diameter and compressed at a 5 mm/min compression speed. Young's modulus and compressive maximum force were calculated from the stress-strain curves recorded using the Trapezium X Texture program (version 1.4.5.).

2.4.3. Porosity and Density Measurements

The porosity (Є) and the density (d) of obtained porous materials were investigated by the liquid displacement method. The graduated cylinder was filled with isopropanol (V_1) as a nonsolvent of used polymers. Samples were weighed (W) and placed in a graduated cylinder. The total volume of isopropanol was recorded after 5 min (V_2). Afterwards, isopropanol-impregnated materials were carefully removed from the cylinder, and the residual isopropanol volume (V_3) was recorded again. Measurements were carried out in triplicate and calculated with the following Equation (1) for porosity and Equation (2) for density:

$$Є\ (\%) = (V_1 - V_3)/(V_2 - V_3) \cdot 100 \quad (1)$$

$$d = W/(V_2 - V_3) \quad (2)$$

2.4.4. Residual Moisture Content

The residual moisture content of porous matrices was analyzed as the weight loss after drying of samples at 105°C for 24 h to a constant weight. Samples (1 cm × 1 cm) were weighed before (W_b) and after (W_a) drying. The measurements were performed in triplicate and calculated as the percentage of the water removed from the samples (Eq. 3):

$$MC\ (\%) = (W_b - W_a)/W_b \cdot 100 \quad (3)$$

2.4.5. Biophysical Skin Parameters Measurements

Instrumental analysis of the biophysical skin parameters was performed after the application of prepared materials. Tewameter (Tewameter TM 300, Courage + Khazaka, Köln, Germany), corneometer (Corneometer CM 825, Courage + Khazaka, Köln, Germany) and colorimeter (Skin-Colorimeter CL 400, Courage + Khazaka, Köln, Germany) were used to analyze skin barrier quality (transepidermal water loss—TEWL), skin surface hydration and skin color, respectively.

Sections (4 × 4 cm) were chosen on the volar forearm skin of both arms of five probands with normal skin (women aged 20–29), with one site serving as the control field. Prepared freeze-dried emulsions and bigels were dissolved in two drops of water directly before spreading to the skin on selected sections. The measurements were carried out before and 15 min, 30 min, 1 h, 2 h, 3 h and 4 h after the application of the samples at a controlled temperature (20–22°C) and humidity (relative humidity 40–60%). The tewametric and colorimetric measurements were investigated in triplicate for each skin site for each proband. However, corneometric results were performed five times at each proband's skin section and are presented as the difference in the corneometer indications between the treated area and the control field at each time point.

2.4.6. Statistical Analysis

One-way ANOVA with Tukey's pairwise was performed using the Past 4.09 program (PAleontological Statistics Software, Oslo, Norway) in order to statistically compare results. Data are shown as the mean ± SD for each experiment with p-values ≤ 0.05 considered significant.

3. Results and Discussion

3.1. Appearance and Structure of Materials

Porous three-dimensional matrices were successfully obtained through freeze-drying of emulsions and bigels formulated using biopolymers, including WPI, sodium alginate, and ethylcellulose (Fig. 2). Mannitol was added as a cryoprotectant, while the oily phase consisted of sea buckthorn oil and beeswax, stabilized by Span 80 as an emulsifier. To enhance the functionality of these materials, chitosan microparticles loaded with *Sambucus nigra* flower extract were incorporated into the materials. However, these microparticles were not distinctly visible in SEM images due to their relatively large size. Nevertheless, their presence was confirmed through broader material imaging, where their dark color from elderflower extract was noticeable and marked with arrows (Fig. 2).

The structural characteristics of the freeze-dried emulsions and bigels differed significantly. While both retained a vivid orange hue due to the presence of sea buckthorn oil, their textural properties and structure varied. The freeze-dried emulsions exhibited a more irregular and linear network, whereas the bigels displayed a more homogenous and uniform structure. Despite these differences, all obtained matrices shared a soft and spongy texture. SEM analysis revealed the intricate architecture of these materials, characterized by an interconnected network of irregular micropores. This porous structure suggests potential applications in fields such as drug delivery and biomaterials, where controlled porosity and structural integrity are crucial factors.

Long-term stability of the developed formulations is a crucial factor determining their applicability as cosmetic or dermatological preparations. The structural and functional integrity of the freeze-dried emulsion and bigel systems was maintained during extended storage indicating their potential for extended shelf life and consistent performance under appropriate storage conditions. The freeze-dried form provided protection against hydrolytic degradation, phase separation, and microbial growth.

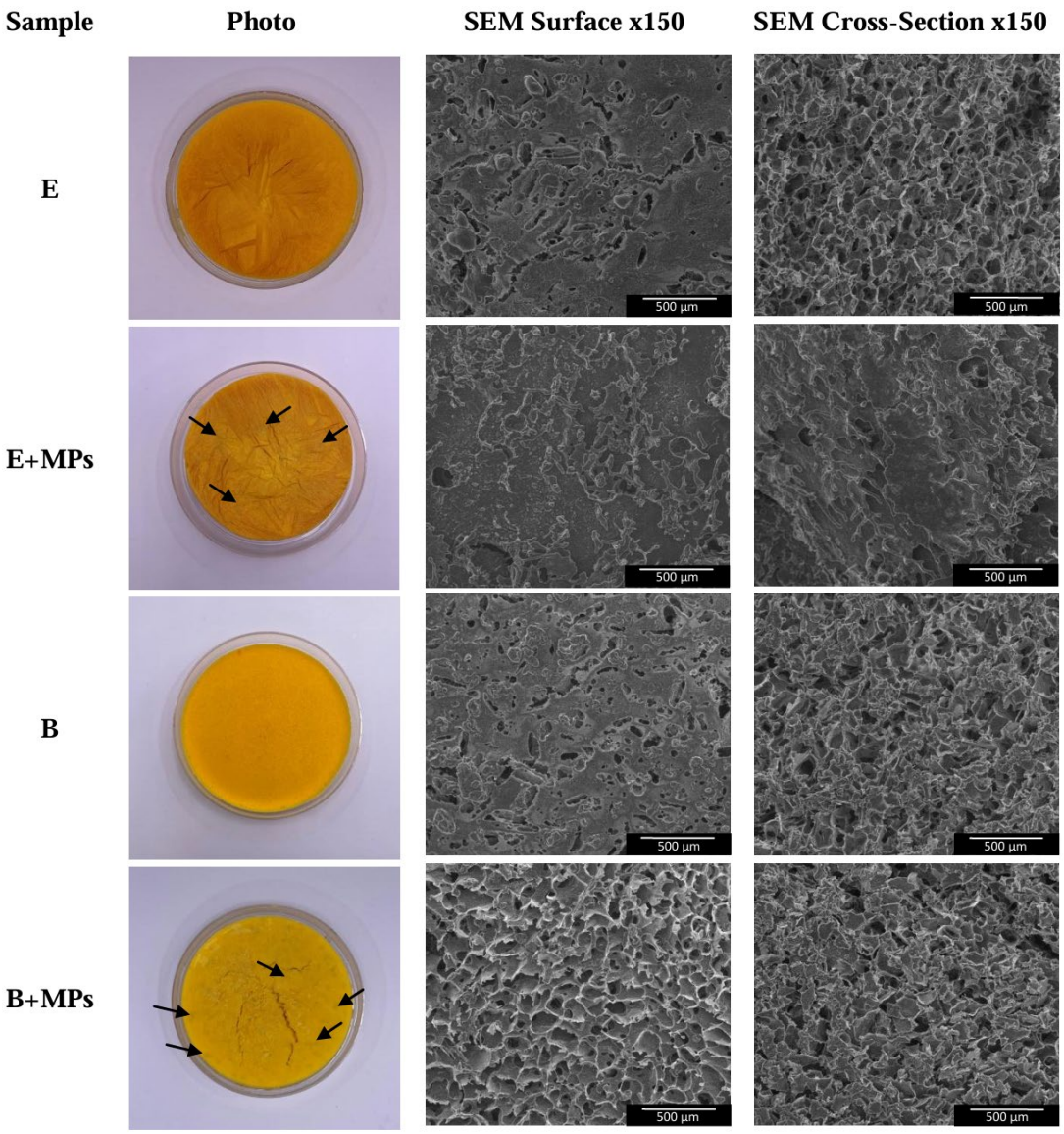


Figure 2. Pictures of obtained freeze-dried materials (the diameter of the container is 60 mm) and SEM images of their surface and cross-section structure in magnification ×150 (scale bar = 500 µm).

Researchers have explored the incorporation of various microparticles into emulsions for enhanced functionality. Chitosan microparticles containing *Eugenia dysenterica* aqueous extract were incorporated into cosmetic emulsions, increasing catechin skin penetration and enhancing angiogenic activity [27]. Moreover, probiotic-loaded alginate microspheres have been used as active ingredients in cosmetic emulsions, effectively protecting and improving the survival of encapsulated probiotic strains [28]. Additionally, microparticles have been shown to stabilize Pickering emulsions [29,30]. *Hypericum perforatum* extract enclosed in lipid carriers has been incorporated into a bigel system composed of Poloxamer 407, borage oil and sorbitan monostearate, designed for wound healing applications [31]. Ketoprofen loaded into poly(vinyl acetate) and hydroxypropyl cellulose carriers have been included in bigel formulations to enhance photostability and ensure the sustained release of the drug while providing analgesic, anti-inflammatory, and antihyperalgesic effects on the skin [32]. Despite these advancements, to our knowledge, no studies have reported on composite materials that integrate encapsulation with freeze-dried emulsions and bigels.

3.2. Mechanical Properties

The mechanical properties of materials composed of biopolymers – WPI, sodium alginate (ALG), and ethylcellulose (EC) – in combination with a cryoprotectant (mannitol), lipids (sea buckthorn oil and beeswax), and an emulsifier (Span 80) were evaluated under compression (Table 1). The study revealed that Young's modulus ranged from approximately 647-777 kPa for emulsions to 1541-1837 kPa for bigels, while the maximum compressive force varied from 9.7 N in emulsions to 12.9 N in bigels. These findings indicate that freeze-dried bigels exhibit significantly greater rigidity and resistance to deformation under applied force compared to emulsions.

Furthermore, the structural integrity and mechanical strength of freeze-dried emulsions and bigels were influenced by their composition. Notably, the incorporation of chitosan microparticles led to an increase in both Young's modulus and the maximum compressive force, suggesting enhanced mechanical stability. This modification may be particularly beneficial for applications requiring materials with improved resistance to mechanical stress. The observed differences in mechanical behavior between emulsions and bigels highlight the importance of formulation strategies in tailoring material properties for specific functional applications.

The combination of biopolymers (WPI, ALG) and lipids (beeswax, sea buckthorn oil) in both bigel and emulsion resulted in strong internal interactions and entangled networks. However, the presence of ethylcellulose in bigels further contributed to mechanical stiffness. The increase in mechanical properties of bigels compared to emulsions may be primarily due to their two structured phases, namely hydrogel and oleogel. This biphasic system created a more interconnected and reinforced network, enhancing rigidity and resistance to deformation. In contrast, emulsions consisting of a single continuous phase with dispersed droplets were more deformable under compression.

Additionally, the incorporation of chitosan microparticles into FD materials might have further strengthened the network by increasing intermolecular interactions, leading to an even greater Young's modulus and compressive force.

Table 1. Mechanical properties of prepared freeze-dried emulsions and bigels with and without the addition of elderflower-loaded chitosan microparticles during compression. Different superscript letters indicate statistically significant differences ($p \leq 0.05$).

Sample	Young's Modulus (kPa)	Compressive Maximum Force (N)
E	647 ± 113^{c}	9.7 ± 0.2^{b}
E+MPs	777 ± 81^{c}	10.4 ± 1.3^{b}
B	1541 ± 118^{b}	12.6 ± 0.4^{a}
B+MPs	1837 ± 93^{a}	12.9 ± 0.7^{a}

The mechanical properties of freeze-dried materials have been studied by other researcher groups. FD scaffolds based on hydroxyapatite-gelatin modified with PLGA microspheres for hard tissue engineering applications had a compressive strength of 1.72 MPa to 2.45 MPa with increasing mechanical strength with a faster freezing rate, resulting in a smaller pore size and thickened crystal walls [33]. Young's modulus of freeze-dried hydrogel scaffolds composed of polyvinyl alcohol and sodium alginate, as a material accelerating wound healing, decreased from 589 kPa to 23 kPa after incorporation of polycaprolactone (PCL) microspheres [13]. Freeze-drying of bigels comprising pectin, chitosan or HPMC resulted in materials for medical uses with increasing hardness ranging from 1.2 N

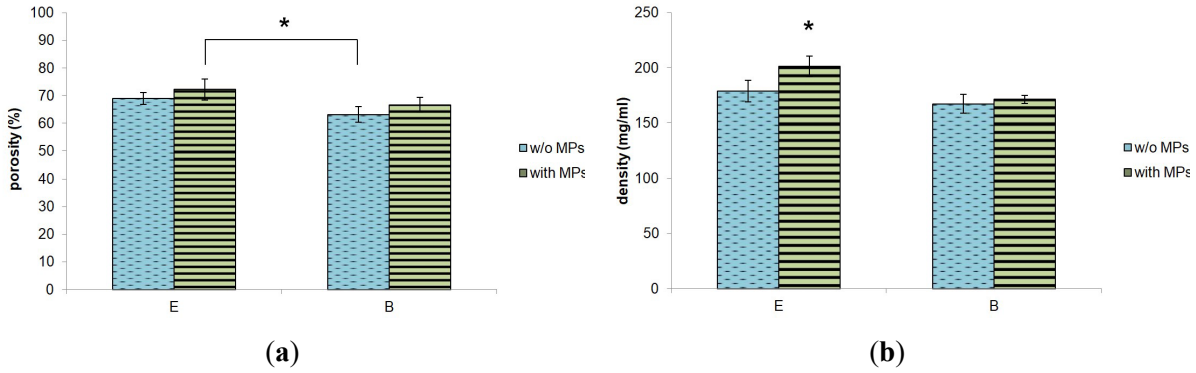
to 6.5 N for samples with higher polymers content [34]. Whereas FD emulsion based on PCL, bovine serum albumin, calcium alginate and hydroxyapatite for tissue engineering displayed compressive strength from ~0.3 MPa to ~0.8 MPa and Young's modulus varying from ~3 MPa to ~10 MPa with both parameters rising after the introduction and further addition of the oily phase due to the increase of thickness of PLC layer adhered onto the pore inner walls [35].

3.3. Porosity and Density Measurements

The porosity and density of FD materials based on biopolymers (WPI, ALG and EC), cryoprotectant (mannitol), lipids (sea buckthorn oil and beeswax) and emulsifier (Span 80) were evaluated through the liquid displacement method using isopropanol. This analysis revealed that FD emulsions exhibited porosity ranging from 69% to 72%, whereas bigels displayed a slightly lower porosity varying from 63% to 67% (Fig. 3a).

The slightly reduced porosity observed in bigels may be attributed to their biphasic structure with the presence of both hydrogel and oleogel phases. The structure of bigels, reinforced by strong interactions between biopolymers and lipids, likely resulted in reduced pore formation during freeze-drying, compared to emulsions.

Interestingly, despite their lower porosity, bigels also exhibited a lower density (167 mg/ml to 171 mg/ml) than emulsions (179 mg/ml to 210 mg/ml) (Fig. 3b). This suggests that bigels, while structurally more rigid, may retain a different internal architecture or phase distribution that affected mass distribution and compaction. The incorporation of extract-loaded chitosan microparticles resulted in a slight increase in both porosity and density across all formulations. This may be due to the microparticles acting as structural fillers that modify the pore network while also increasing mass per unit volume.



(**a**) (**b**)

Figure 3. Porosity (a) and density (b) of prepared freeze-dried emulsions (E) and bigels (B) with and without the addition of elderflower-loaded chitosan microparticles (MPs). Significant differences ($p \leq 0.05$) between samples were marked on the graph with (*).

Porosity and density measurement results are comparable with those reported by other researchers. FD composites based on silk fibroin/collagen/hyaluronic acid scaffolds and PLGA microspheres for cartilage tissue engineering had porosity ranging from 45% to 59% [36]. Poly(L-lactic acid) microsphere-loaded FD emulsion based on calcium alginate and hydroxyapatite had 75-90% porosity [37]. Freeze-dried PCL, bovine serum albumin, and calcium alginate emulsion modified with hydroxyapatite had ~81-91% porosity [35]. The porosity of freeze-dried hydrogels decreased from 85% to 80% with increased concentration of alginate [38] and increased from 39% to 88% with higher

amounts of xanthan gum added into whey protein concentrate hydrogel [39]. The density of FD emulsion based on epoxy/cellulose (65 to 150 mg/ml) [40] and tea oil/maltodextrin/xanthan gum/lysozyme nanoparticles (120-340 mg/ml) [41] have also been investigated. In comparison, WPI FD hydrogel had ~220 mg/ml density [42].

3.4. Residual Moisture Content

Results of residual moisture content after freeze-drying of emulsions and bigels were investigated as the percentage of weight loss during drying samples to a constant weight (Fig. 4). Moisture content varied across formulations, with the FD emulsion containing 4.2%, while the addition of microparticles increased it to 7.1%. Bigels had a higher residual moisture content of 7.5%, but its value decreased to 5.7% when combined with microparticles. These variations may be attributed to differences in water-binding capacity, phase organization, and microparticle interactions. The higher moisture content in bigels could result from their two structured phases, which retain water more effectively. However, the unexpected reduction in moisture for bigels with microparticles suggests that chitosan may facilitate more efficient water removal during freeze-drying. These variations in moisture content highlight the influence of formulation and additives on the water-holding capacity of FD materials, which can impact their stability and performance in various applications.

Other studies have also examined the residual moisture content of freeze-dried materials. Emulsions prepared from xanthan gum mixed with maltodextrin and lysozyme nanoparticles in aqueous phase and tea oil were reported to have ~0.7-3.5% moisture content with higher values ascribed to stronger hydrogen bonds within samples [41]. Black chokeberry pomace-loaded emulsions exhibited approximately 1.6-1.9% moisture content after freeze-drying [43]. The moisture content of FD samples composed of WPI and alginate varied from 3.9% to 4.3% [44].

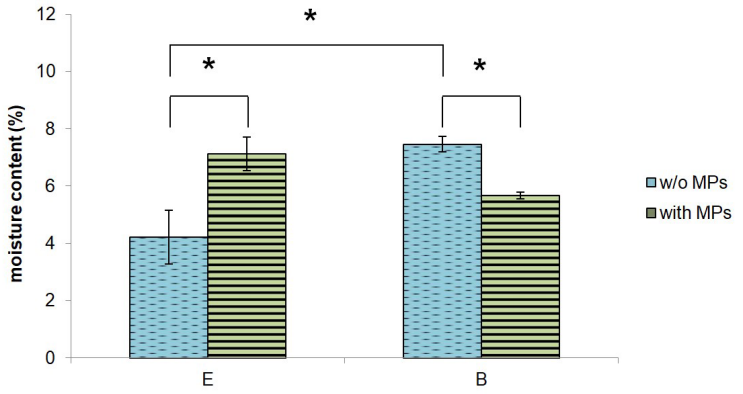


Figure 4. The moisture content of prepared freeze-dried emulsions (E) and bigels (B) with and without the addition of elderflower-loaded chitosan microparticles (MPs). Significant differences ($p \leq 0.05$) between samples are marked on the graph with (*).

3.5. Biophysical Skin Parameters Measurements

Biophysical skin parameters, including skin surface hydration, skin barrier quality (transepidermal water loss – TELW) and skin color, were assessed with the use of Courage+Khazaka probes. The obtained freeze-dried emulsions and bigels, with and without the addition of elderflower-loaded chitosan microparticles, were topically applied to the probands' skin and reconstructed back to emulsion or bigel with water during the spreading of materials on the skin.

The hydration level of the *stratum corneum* serves as an indirect indicator of overall skin moisture. A corneometer measures hydration at a very shallow depth, focusing solely on the outermost skin layer to prevent interference from deeper skin layers. Since the device relies on electrical capacitance, the presence of substances like salts or residues from applied products on the skin surface has little impact on the accuracy of the readings.

According to the obtained results (Fig. 5), it is evident that the topical treatment with all tested materials led to an increase in the hydration of the *stratum corneum*. The highest rise in water content in the outermost skin layer was observed after applying bigel modified with extract-loaded microparticles, while the emulsion containing microparticles demonstrated the weakest skin-hydration effect. Fifteen minutes after treatment, the skin hydration levels in areas treated with the emulsion and emulsion with microparticles were 8.6 a.u. and 6.3 a.u., respectively. In comparison, bigel and bigel with microparticles resulted in hydration levels of 7.9 a.u. and 11.8 a.u., respectively. After an additional 15 minutes, the hydration levels decreased to a range of 4.2–5.2 a.u., with the highest value of 9.3 a.u. recorded for bigel with microparticles. One and two hours after application, the hydration values ranged from 4.9 to 7.3 a.u. and from 5.3 to 9.7 a.u., respectively. After three hours, skin areas treated with bigels exhibited higher hydration levels (8.5–9.0 a.u.) compared to emulsions (5.8–6.1 a.u.). At the final measurement point, the *stratum corneum* hydration levels ranged from 8.0 to 9.7 a.u. for areas treated with emulsion and bigels, while the skin treated with the emulsion containing extract-loaded microparticles showed a lower value of 5.9 a.u.

The enhanced skin hydration observed after applying the freeze-dried emulsions and bigels can be attributed to the synergistic effect of their carefully selected components. Sodium alginate and WPI form a protective film on the skin, enhancing moisture retention [45,46]. Furthermore, the composition of WPI is similar to Natural Moisturizing Factor (NMF) naturally present in the *stratum corneum*. They both are rich in amino acids like serine, glycine, and proline, which can enhance skin hydration by supplying building blocks for NMF and improving moisture retention. Therefore, WPI may support the skin's natural hydration process by rebuilding the NMF. Mannitol acts as a cryoprotectant, preserving the biopolymer matrix's structural integrity and thus may improve its rehydration capacity [47], while sea buckthorn oil and beeswax indirectly moisturize the skin [48,49]. Chitosan microparticles containing *Sambucus nigra* flower extract may contribute to the observed moisturizing effect due to the presence of flavonoids and phenolic compounds with known antioxidant and hydrating properties [50]. The superior hydration effect of bigel modified with microparticles may result from the structured network of the biopolymer-lipid matrix, which enhances water retention and prolongs the moisturizing effect.

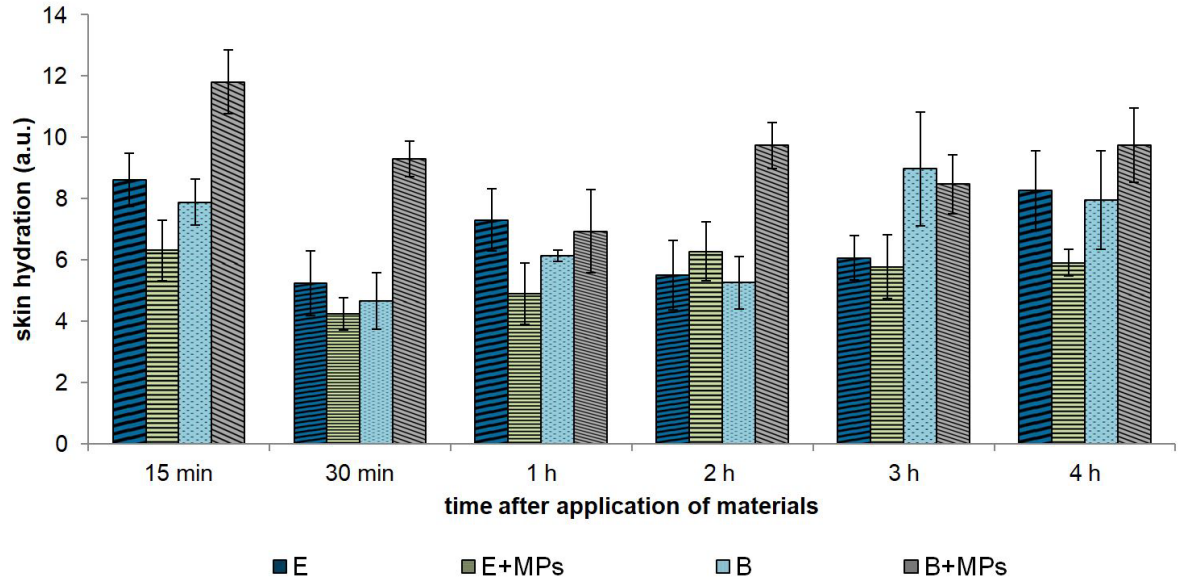


Figure 5. Corneometric skin measurements after topical application of prepared freeze-dried emulsions (E) and bigels (B) with and without the addition of elderflower-loaded chitosan microparticles (MPs). The results show differences in corneometer indications between the treated and untreated control areas at each measured interval.

The evaporation of water from the skin is a natural aspect of the body's metabolism. TEWL serves as an indicator of the skin's barrier integrity, as any disruption in this function leads to an increase in water loss. Thus, measuring TEWL is essential for determining the effectiveness of topical products.

Tewametric measurements indicate that the application of all prepared samples led to a reduction in TEWL, thereby enhancing the epidermal permeability barrier function and improving overall skin barrier integrity (Fig. 6). TEWL values significantly decreased from 11.3–12.4 g/h/m² to 10.1–12.0 g/h/m², suggesting that the formulations strengthened the skin's protective barrier. This improvement can be attributed to the combined action of biopolymers and lipids, which form a protective film on the skin surface, reducing transepidermal water loss. Additionally, the moisturizing properties of *Sambucus nigra* flower extract-loaded chitosan microparticles, along with the occlusive effect of beeswax and sea buckthorn oil, contribute to improved water retention and barrier repair, resulting in enhanced skin hydration and reduced TEWL. Furthermore, the composition of sea buckthorn oil is similar to that of the skin's natural lipids, particularly essential fatty acids like omega-3, omega-6, omega-7, and omega-9, as well as lipid-soluble vitamins [51,52]. These lipids can integrate into the skin's lipids, supporting hydration and barrier repair, which is shown as reduced TEWL.

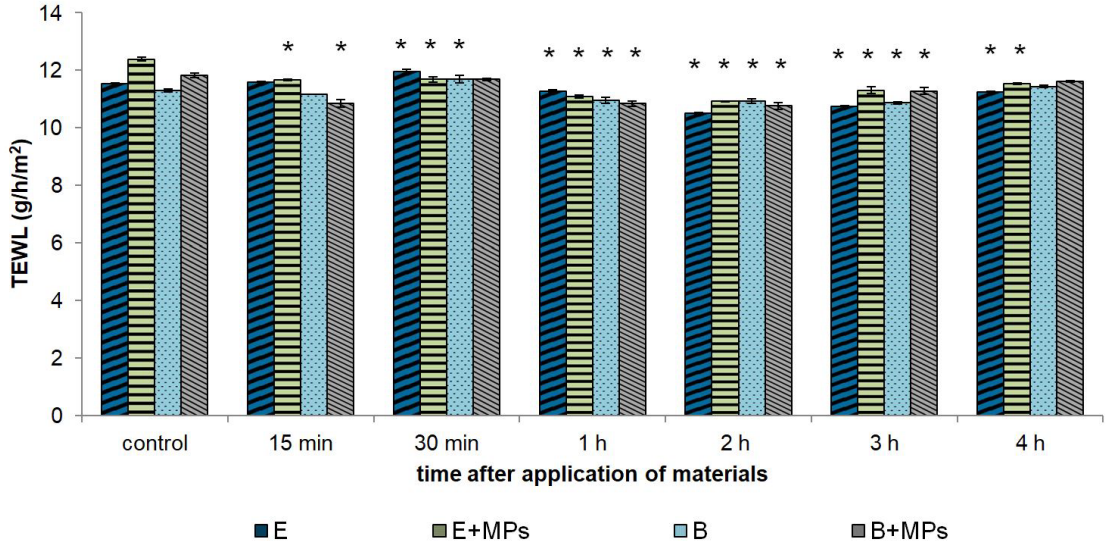


Figure 6. Tewametric measurements of skin before (control) and after topical application of prepared freeze-dried emulsions (E) and bigels (B) with and without the addition of elderflower-loaded chitosan microparticles (MPs). * indicates a difference at $p < 0.05$ between the results at an appropriate time compared to those made for the control field.

The L*a*b* color space coordinates are used to represent skin color measurements. The L* value indicates skin brightness; the a* value represents the position on the red-green axis, and it is used to assess skin redness, microcirculation, and erythema, while the b* value corresponds to the blue-yellow axis (skin pigmentation).

Based on colorimeter indications (Fig. 7), all of the formulated samples led to a reduction in skin redness, with values decreasing from 7.25–7.68 a.u. before the application of the materials to 4.28–6.62 a.u. following the application of freeze-dried emulsions and bigels, both with and without the addition of chitosan microparticles containing *Sambucus nigra* flower extract. This reduction in redness can be attributed to sea buckthorn, which has been shown to exhibit decreasing melanin content and skin erythema properties [53]. Moreover, biopolymers (sodium alginate, WPI, ethylcellulose), lipids (sea buckthorn oil, beeswax), and cryoprotectant (mannitol) likely played a key role in soothing the skin, reducing inflammation, and improving skin tone. These components formed a protective barrier that helped calm the skin, diminish erythema, and promote a more even complexion. In addition, the soothing and anti-inflammatory effects of the herbal extract and chitosan might have helped to calm the skin and reduce erythema, leading to a more even skin tone. The active compounds in the formulations, such as flavonoids and phenolic compounds, may have contributed to reducing the skin's redness by targeting inflammatory pathways and promoting a more even complexion.

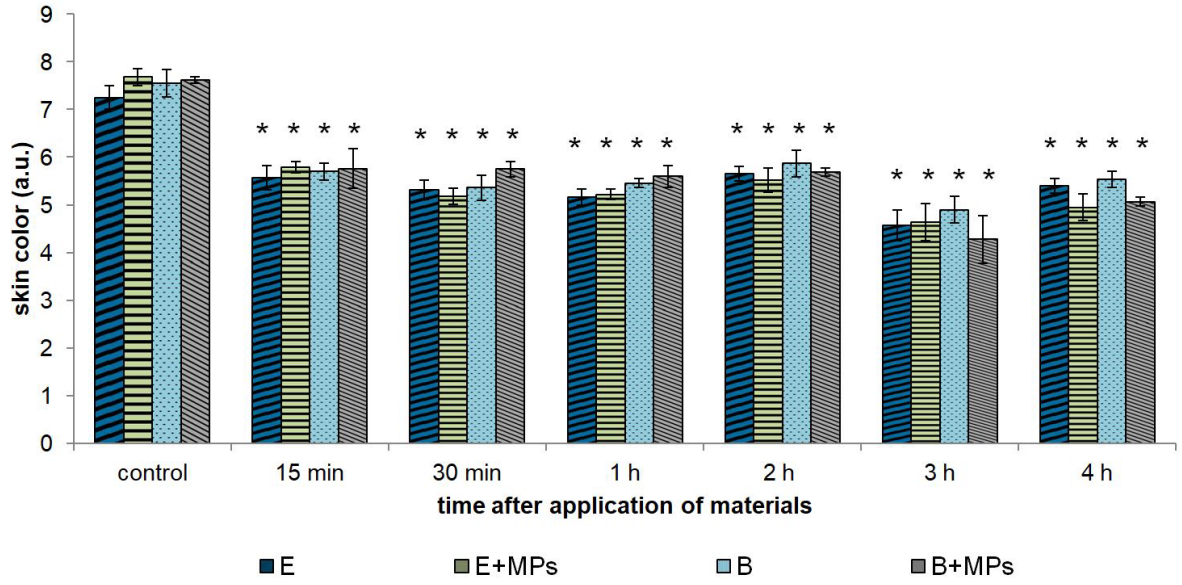


Figure 7. Colorimetric measurements of skin before (control) and after topical application of prepared freeze-dried emulsions (E) and bigels (B) with and without the addition of elderflower-loaded chitosan microparticles (MPs). * indicates a difference at $p < 0.05$ between the results at an appropriate time compared to those made for the control field.

3.6. General Discussion

The development of freeze-dried emulsions and bigels incorporating chitosan microparticles loaded with *Sambucus nigra* extract represents a novel approach that may combine the structural advantages of biopolymer networks with the functional benefits of controlled delivery systems. The physicochemical and mechanical characteristics of the obtained materials likely depend on the composition and interactions among the polymers, lipid components, and microparticles. The co-assembly of WPI, ALG, and EC may give rise to multicomponent polymeric networks in which distinct but complementary gelation mechanisms operate simultaneously. WPI, a globular protein containing reactive carboxyl, amino, and sulfhydryl groups, might undergo partial unfolding and rearrangement during formulation, which could facilitate intermolecular hydrogen bonding and electrostatic association with the carboxylate and hydroxyl groups of sodium alginate [54]. Such interactions may contribute to the formation of a cohesive protein-polysaccharide network. In parallel, EC may form the oleogel phase through hydrophobic association, chain entanglement, and partial crystallization within lipid-rich domains, thereby reinforcing the overall matrix [55,56]. The inclusion of beeswax and sea buckthorn oil could further stabilize the oleogel network by promoting van der Waals interactions and molecular packing within the lipophilic domains, leading to a more structurally coherent biphasic system [55].

The observed correlation between gel formation and physicochemical properties suggests that differences in molecular-level interactions drive the distinct structural and functional behaviors of the materials. Freeze-dried emulsions exhibited a more irregular pore network, which may indicate less cohesive interfacial stabilization, whereas bigels displayed a more compact and uniform microstructure that might result from stronger connectivity between the hydrogel and oleogel phases. The presence of mannitol as a cryoprotectant likely contributed to maintaining hydrogen-bonded networks during

freezing, thereby reducing ice crystal formation and preserving the pore architecture upon freeze-drying [57].

The incorporation of chitosan microparticles may also influence the morphology and functional properties of freeze-dried materials. The presence of positively charged chitosan microparticles could interact electrostatically with negatively charged alginate chains or the protein backbone, potentially leading to partial reinforcement of the polymeric matrix [58,59]. At the same time, these microparticles might act as reservoirs for the phenolic constituents from *Sambucus nigra* extract. The interactions between chitosan and extract components, such as phenolic acids or flavonoids, could further contribute to network stability through hydrogen bonding [60,61]. The microparticles matrix may protect phenolic compounds from degradation during processing and storage, ensuring their availability at the moment of topical application. The presence of chitosan and bioactive plant extract may explain the improved cutaneous hydration and reduced TEWL observed after application of these materials. The skin analyses indicated that all prepared materials contribute to improved barrier function, possibly due to the synergistic effects of the hydrophilic-lipophilic components and the antioxidant-rich microparticles.

From a broader perspective, these findings suggest that design of composite polymeric systems may support the development of sustainable cosmetic formulations with reduced water consumption. The possibility of reconstituting freeze-dried emulsions or bigels with minimal water immediately before application could significantly reduce water use in both manufacturing (freeze-drying offers the possibility of recovering and reusing sublimed water) and end-use, aligning with green chemistry principles. Furthermore, the integration of biodegradable polymers and natural extracts may offer an eco-friendly approach to developing high-performance materials for dermatological and cosmetic applications.

4. Conclusions

The study successfully developed freeze-dried emulsions and bigels modified with chitosan microparticles loaded with *Sambucus nigra* flower extract, demonstrating the enhanced cutaneous effect. The combination of biopolymers (WPI and sodium alginate) and cryoprotectant (mannitol) in the aqueous phase, lipids (sunflower oil and beeswax) and emulsifier (Span 80) in the oily phase of the emulsion and additionally ethylcellulose in bigel created a stable, porous structure after freeze-drying. FD bigel exhibited higher values of Young's modulus and residual moisture content, whereas FD emulsion presented higher porosity and density. Eco-friendly, three-dimensional materials with a porous structure containing polymeric microparticles with encapsulated plant extract (flowers of *Sambucus nigra)* were designed to dissolve in a minimal amount of aqueous solvent back to emulsion or bigel immediately before its application to the skin. Active substances from elderflower extract showing antioxidant properties and a positive effect on the skin condition were protected in chitosan microparticles during the freeze-drying process and released at the moment of the cosmetic application to the skin (during spreading on the skin). The application of fabricated materials significantly increased the hydration of the *stratum corneum* and decreased skin redness and TEWL, indicating improved skin barrier quality, moistened and soothed skin due to the materials components. Therefore, the combination of microencapsulation and sponge-like materials resulted in the development of an innovative eco-friendly material form that is effective and suitable for cosmetic and dermatological applications. However, expanding skin penetration studies and exploring additional bioactive compounds for broader dermatological and biomedical applications should be further explored.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest: The authors declare no conflict of interest.

References

[1] M. Lengyel, N. Kállai-Szabó, V. Antal, A.J. Laki, I. Antal, Microparticles, Microspheres, and Microcapsules for Advanced Drug Delivery, Sci. Pharm. 2019, Vol. 87, Page 20 87 (2019) 20. https://doi.org/10.3390/SCIPHARM87030020.

[2] K. Huang, Y. Yuan, X. Baojun, A Critical Review on the Microencapsulation of Bioactive Compounds and Their Application, Food Rev. Int. 39 (2023). https://doi.org/10.1080/87559129.2021.1963978.

[3] M. Hamdi, H. Sun, L. Pan, D. Wang, M. Sun, Z. Zeng, S. Li, Q. Dong, F. Su, Chitosan and its derivatives as potential biomaterials for biomedical and pharmaceutical applications: A comprehensive review on green extraction approaches, recent progresses, and perspectives, Eur. Polym. J. 229 (2025) 113882. https://doi.org/10.1016/J.EURPOLYMJ.2025.113882.

[4] W. Szczęsna, M. Tsirigotis-Maniecka, L. Szyk-Warszyńska, S. Balicki, P. Warszyński, K.A. Wilk, Insight into multilayered alginate/chitosan microparticles for oral administration of large cranberry fruit extract, Eur. Polym. J. 160 (2021) 110776. https://doi.org/10.1016/J.EURPOLYMJ.2021.110776.

[5] F. Kazemi-Andalib, M. Mohammadikish, A. Divsalar, U. Sahebi, Hollow microcapsule with pH-sensitive chitosan/polymer shell for in vitro delivery of curcumin and gemcitabine, Eur. Polym. J. 162 (2022). https://doi.org/10.1016/j.eurpolymj.2021.110887.

[6] G.X. Wang, D. Huang, J.H. Ji, C. Völker, F.R. Wurm, Seawater-Degradable Polymers—Fighting the Marine Plastic Pollution, Adv. Sci. 8 (2021) 1–26. https://doi.org/10.1002/advs.202001121.

[7] G.I. Edo, A.N. Mafe, N.F. Razooqi, E.C. Umelo, T.S. Gaaz, E.F. Isoje, U.A. Igbuku, P.O. Akpoghelie, R.A. Opiti, A.E.A. Essaghah, D.S. Ahmed, H. Umar, D.U. Ozsahin, Advances in bio-polymer coatings for probiotic microencapsulation: chitosan and beyond for enhanced stability and controlled release, Des. Monomers Polym. 28 (2025) 1–34. https://doi.org/10.1080/15685551.2024.2448122.

[8] G. Ozkan, P. Franco, I. De Marco, J. Xiao, E. Capanoglu, A review of microencapsulation methods for food antioxidants: Principles, advantages, drawbacks and applications, Food Chem. 272 (2019) 494–506. https://doi.org/10.1016/J.FOODCHEM.2018.07.205.

[9] D.J. McClements, Encapsulation, protection, and delivery of bioactive proteins and peptides using

nanoparticle and microparticle systems: A review, Adv. Colloid Interface Sci. 253 (2018) 1–22. https://doi.org/10.1016/j.cis.2018.02.002.

[10] K. Młynarczyk, D. Walkowiak-Tomczak, G.P. Łysiak, Bioactive properties of Sambucus nigra L. as a functional ingredient for food and pharmaceutical industry, J. Funct. Foods 40 (2018) 377–390. https://doi.org/10.1016/J.JFF.2017.11.025.

[11] V. Milena, M. Tatjana, Z. Gökhan, B. Ivana, C. Aleksandra, M.F. Mohammad, R. Marija, Advantages of contemporary extraction techniques for the extraction of bioactive constituents from black elderberry (Sambucus nigra L.)flowers, Ind. Crops Prod. 136 (2019). https://doi.org/10.1016/j.indcrop.2019.04.058.

[12] S.S. Ferreira, A.M. Silva, F.M. Nunes, Sambucus nigra L. Fruits and Flowers: Chemical Composition and Related Bioactivities, Food Rev. Int. 00 (2020) 1–29. https://doi.org/10.1080/87559129.2020.1788578.

[13] M. Bahadoran, A. Shamloo, Y.D. Nokoorani, Development of a polyvinyl alcohol/sodium alginate hydrogel-based scaffold incorporating bFGF-encapsulated microspheres for accelerated wound healing, Sci. Rep. 10 (2020) 7–9. https://doi.org/10.1038/s41598-020-64480-9.

[14] C. Wang, W. Luo, P. Li, S. Li, Z. Yang, Z. Hu, Y. Liu, N. Ao, Preparation and evaluation of chitosan/alginate porous microspheres/Bletilla striata polysaccharide composite hemostatic sponges, Carbohydr. Polym. 174 (2017). https://doi.org/10.1016/j.carbpol.2017.06.112.

[15] F.F.O. Sousa, A. Luzardo-Álvarez, A. Pérez-Estévéz, R. Seoane-Prado, J. Blanco-Méndez, Sponges containing tetracycline loaded-PLGA-zein microparticles as a periodontal controlled release device, J. Drug Deliv. Sci. Technol. 59 (2020). https://doi.org/10.1016/j.jddst.2020.101858.

[16] M.D. Figueroa-Pizano, I. Vélaz, F.J. Peñas, P. Zavala-Rivera, A.J. Rosas-Durazo, A.D. Maldonado-Arce, M.E. Martínez-Barbosa, Effect of freeze-thawing conditions for preparation of chitosan-poly (vinyl alcohol) hydrogels and drug release studies, Carbohydr. Polym. 195 (2018) 476–485. https://doi.org/10.1016/j.carbpol.2018.05.004.

[17] S.R. Pardeshi, N.S. Deshmukh, D.R. Telange, S.N. Nangare, Y.Y. Sonar, S.H. Lakade, M.T. Harde, C. V. Pardeshi, A. Gholap, P.K. Deshmukh, M.P. More, Process development and quality attributes for the freeze-drying process in pharmaceuticals, biopharmaceuticals and nanomedicine delivery: a state-of-the-art review, Futur. J. Pharm. Sci. 9 (2023). https://doi.org/10.1186/s43094-023-00551-8.

[18] C. Wang, A. Killpatrick, A. Humphrey, M. Guo, Whey protein functional properties and applications in food formulation, Whey Protein Prod. Chem. Funct. Appl. (2019) 157–204. https://doi.org/10.1002/9781119256052.ch7.

[19] J.P.C.M. Peters, F.J. Vergeldt, H. Van As, H. Luyten, R.M. Boom, A.J. van der Goot, Unravelling of the water-binding capacity of cold-gelated whey protein microparticles, Food Hydrocoll. 63 (2017) 533–544. https://doi.org/10.1016/j.foodhyd.2016.09.038.

[20] B. Jadach, W. Świetlik, A. Froelich, Sodium Alginate as a Pharmaceutical Excipient: Novel Applications of a Well-known Polymer, J. Pharm. Sci. 111 (2022) 1250–1261. https://doi.org/10.1016/J.XPHS.2021.12.024.

[21] P. Ahmadi, A. Jahanban-Esfahlan, A. Ahmadi, M. Tabibiazar, M. Mohammadifar, Development of Ethyl Cellulose-based Formulations: A Perspective on the Novel Technical Methods, Food Rev. Int. 38 (2022) 685–732. https://doi.org/10.1080/87559129.2020.1741007.

[22] W. Prus-Walendziak, J. Kozlowska, Lyophilized emulsions in the form of 3d porous matrices as a novel material for topical application, Materials (Basel). 14 (2021) 1–18. https://doi.org/10.3390/ma14040950.

[23] W. Walendziak, T.E.L. Douglas, J. Kozlowska, Physicochemical Properties of Freeze-Dried Bigel-Based Materials Composed of Sodium Alginate/Whey Protein Isolate Hydrogel and Ethylcellulose/Sunflower

Oil Oleogel, Biomacromolecules 26 (2025) 2344–2355. https://doi.org/10.1021/acs.biomac.4c01677.

[24] N. Stachowiak, J. Kowalonek, J. Kozlowska, materials Freeze-Dried Matrices Composed of Degradable Polymers with Surfactant-Loaded Microparticles Based on Pectin and Sodium Alginate, (2021). https://doi.org/10.3390/ma14113044.

[25] J. Kozlowska, W. Prus-Walendziak, N. Stachowiak, A. Bajek, L. Kazmierski, B. Tylkowski, Modification of collagen/gelatin/hydroxyethyl cellulose-based materials by addition of herbal extract-loaded microspheres made from gellan gum and xanthan gum, Materials (Basel). 13 (2020) 1–15. https://doi.org/10.3390/MA13163507.

[26] A. Magiera, M. Kuźnia, W. Jerzak, Analysis of the Structural, Chemical, and Mechanical Characteristics of Polyurethane Foam Infused with Waste from Thermal Processing, Materials (Basel). 18 (2025). https://doi.org/10.3390/ma18061327.

[27] S.M. Mazutti da Silva, R. Ferreira-Nunes, C.R. Amore, D.H.N. Martins, A. Pic-Taylor, Y.M. Fonseca-Bazzo, D. Silveira, G.M. Gelfuso, P.O. Magalhães, Emulsion incorporating Eugenia dysenterica aqueous extract entrapped in chitosan microparticles as a novel topical treatment of cutaneous infections, J. Drug Deliv. Sci. Technol. 55 (2020) 101372. https://doi.org/10.1016/j.jddst.2019.101372.

[28] A. Łętocha, A. Michalczyk, P. Ostrowska, M. Miastkowska, E. Sikora, Probiotics-Loaded Microspheres for Cosmetic Applications, Appl. Sci. 14 (2024). https://doi.org/10.3390/app14031183.

[29] X. Yang, Y. Wang, R. Bai, H. Ma, W. Wang, H. Sun, Y. Dong, F. Qu, Q. Tang, T. Guo, B.P. Binks, T. Meng, Pickering emulsion-enhanced interfacial biocatalysis: Tailored alginate microparticles act as particulate emulsifier and enzyme carrier, Green Chem. 21 (2019). https://doi.org/10.1039/c8gc03573c.

[30] S. Liu, Y. Zhu, Y. Wu, A. Lue, C. Zhang, Hydrophobic modification of regenerated cellulose microparticles with enhanced emulsifying capacity for O/W Pickering emulsion, Cellulose 26 (2019). https://doi.org/10.1007/s10570-019-02538-2.

[31] Y. Sotirova, V. Gugleva, S. Stoeva, I. Kolev, R. Nikolova, M. Marudova, K. Nikolova, Y. Kiselova-Kaneva, M. Hristova, V. Andonova, Bigel Formulations of Nanoencapsulated St. John's Wort Extract—An Approach for Enhanced Wound Healing, Gels 9 (2023). https://doi.org/10.3390/gels9050360.

[32] V. Andonova, P. Peneva, G.S. Georgiev, V.T. Toncheva, E. Apostolova, Z. Peychev, S. Dimitrova, M. Katsarova, N. Petrova, M. Kassarova, Ketoprofen-loaded polymer carriers in bigel formulation: An approach to enhancing drug photostability in topical application forms, Int. J. Nanomedicine 12 (2017) 6221–6238. https://doi.org/10.2147/IJN.S140934.

[33] F. Ghorbani, H. Nojehdehian, A. Zamanian, Physicochemical and mechanical properties of freeze cast hydroxyapatite-gelatin scaffolds with dexamethasone loaded PLGA microspheres for hard tissue engineering applications, Mater. Sci. Eng. C 69 (2016) 208–220. https://doi.org/10.1016/j.msec.2016.06.079.

[34] A. Martín-Illana, F. Notario-Pérez, R. Cazorla-Luna, R. Ruiz-Caro, M.D. Veiga, Smart Freeze-Dried Bigels for the Prevention of the Sexual Transmission of HIV by Accelerating the Vaginal Release of Tenofovir during Intercourse, Pharm. 2019, Vol. 11, Page 232 11 (2019) 232. https://doi.org/10.3390/PHARMACEUTICS11050232.

[35] Y. Hu, W. Han, Y. Chen, R. Zou, Y. Ouyang, W. Zhou, Z. Yang, C. Wang, One-Pot Fabrication of Poly(ε-Caprolactone)-Incorporated Bovine Serum Albumin/Calcium Alginate/Hydroxyapatite Nanocomposite Scaffolds by High Internal Phase Emulsion Templates, Macromol. Mater. Eng. 302 (2017) 1–10. https://doi.org/10.1002/mame.201600367.

[36] J. Wang, X. Sun, Z. Zhang, Y. Wang, C. Huang, C. Yang, L. Liu, Q. Zhang, Silk

fibroin/collagen/hyaluronic acid scaffold incorporating pilose antler polypeptides microspheres for cartilage tissue engineering, Mater. Sci. Eng. C 94 (2019) 35–44. https://doi.org/10.1016/j.msec.2018.09.017.

[37] Y. Hu, S. Ma, Z. Yang, W. Zhou, Z. Du, J. Huang, H. Yi, C. Wang, Facile fabrication of poly(L-lactic acid) microsphere-incorporated calcium alginate/hydroxyapatite porous scaffolds based on Pickering emulsion templates, Colloids Surfaces B Biointerfaces 140 (2016) 382–391. https://doi.org/10.1016/j.colsurfb.2016.01.005.

[38] N. Bahrami, A. Farzin, F. Bayat, A. Goodarzi, M. Salehi, R. Karimi, A. Mohamadnia, A. Parhiz, J. Ai, Optimization of 3D Alginate Scaffold Properties with Interconnected Porosity Using Freeze-drying Method for Cartilage Tissue Engineering Application, Arch. Neurosci. 6 (2019) 4–11. https://doi.org/10.5812/ans.85122.

[39] M. Pilevaran, H. Tavakolipour, S. Naji-Tabasi, A.H. Elhamirad, Investigation of functional, textural, and thermal properties of soluble complex of whey protein–xanthan gum hydrogel, J. Food Process Eng. 44 (2021). https://doi.org/10.1111/jfpe.13751.

[40] J. Li, Q. Yan, Z. Cai, Fabrication and characterization of emulsified and freeze-dried epoxy/cellulose nanofibril nanocomposite foam, Cellulose 26 (2019) 1769–1780. https://doi.org/10.1007/s10570-018-2164-x.

[41] W. Xu, H. Sun, H. Li, Z. Li, S. Zheng, D. Luo, Y. Ning, Y. Wang, B.R. Shah, Preparation and characterization of tea oil powder with high water solubility using Pickering emulsion template and vacuum freeze-drying, LWT 160 (2022) 113330. https://doi.org/10.1016/J.LWT.2022.113330.

[42] L. Manzocco, S. Plazzotta, J. Powell, A. de Vries, D. Rousseau, S. Calligaris, Structural characterisation and sorption capability of whey protein aerogels obtained by freeze-drying or supercritical drying, Food Hydrocoll. 122 (2021). https://doi.org/10.1016/j.foodhyd.2021.107117.

[43] V. Eisinaitė, D. Leskauskaitė, M. Pukalskienė, P.R. Venskutonis, Freeze-drying of black chokeberry pomace extract–loaded double emulsions to obtain dispersible powders, J. Food Sci. 85 (2020) 628–638. https://doi.org/10.1111/1750-3841.14995.

[44] R. Rajam, P. Karthik, S. Parthasarathi, G.S. Joseph, C. Anandharamakrishnan, Effect of whey protein - alginate wall systems on survival of microencapsulated Lactobacillus plantarum in simulated gastrointestinal conditions, J. Funct. Foods 4 (2012) 891–898. https://doi.org/10.1016/j.jff.2012.06.006.

[45] K.G. Ramawat, J.M. Mérillon, Polysaccharides: Bioactivity and biotechnology, Polysaccharides Bioactivity Biotechnol. (2015) 1–2241. https://doi.org/10.1007/978-3-319-16298-0.

[46] D. Sobkowska, I. Micek, M. Urbańska, A. Seraszek-Jaros, G. Nowak, L. Zaprutko, R. Czajkowski, Z. Adamski, J. Gornowicz-Porowska, The effects of baths and wet wraps with a sweet whey solution on the level of hydration and barrier function of the epidermis, Postep. Dermatologii i Alergol. 38 (2021) 798–772. https://doi.org/10.5114/ada.2021.110078.

[47] C.Y. Wong, H. Al-Salami, C.R. Dass, Lyophilisation Improves Bioactivity and Stability of Insulin-Loaded Polymeric-Oligonucleotide Nanoparticles for Diabetes Treatment, AAPS PharmSciTech 21 (2020) 1–20. https://doi.org/10.1208/s12249-020-01648-6.

[48] J. Pavlačková, P. Egner, R. Slavík, P. Mokrejš, R. Gál, Hydration and barrier potential of cosmetic matrices with bee products, Molecules 25 (2020) 1–13. https://doi.org/10.3390/molecules25112510.

[49] B.A. Khan, N. Akhtar, Hippophae rhamnoides oil-in-water (O/W) emulsion improves barrier function in healthy human subjects, Pak. J. Pharm. Sci. 27 (2014) 1919–1922.

[50] P. Ferreira-Santos, H. Badim, Â.C. Salvador, A.J.D. Silvestre, S.A.O. Santos, S.M. Rocha, A.M. Sousa,

M.O. Pereira, C.P. Wilson, C.M.R. Rocha, J.A. Teixeira, C.M. Botelho, Chemical characterization of sambucus Nigra L. Flowers aqueous extract and its biological implications, Biomolecules 11 (2021) 1222. https://doi.org/10.3390/BIOM11081222/S1.

[51] B.A. Khan, N. Akhtar, T. Mahmood, H.M.S. Khan, S.U. Zaman, A. Rasul, M. Iqbal, A. Ali, S. Khan, M. Qayum, In-vivo study of stratum corneum water content and transepideramal water loss using a newly formulated topical cream of hippophae rhamnoides fruit extract, African J. Pharm. Pharmacol. 5 (2011) 1092–1095.

[52] N. Poljšak, S. Kreft, N. Kočevar Glavač, Vegetable butters and oils in skin wound healing: Scientific evidence for new opportunities in dermatology, Phyther. Res. 34 (2020) 254–269. https://doi.org/10.1002/ptr.6524.

[53] R. Kausar, N. Akhtar, H.M.S. Khan, M.H. Kiani, Effects of flavonoids and antioxidants of blackberry on skin whitening and erythema, Asian J. Chem. 29 (2017) 1365–1368. https://doi.org/10.14233/ajchem.2017.20510.

[54] X. Liu, X. Qin, Y. Wang, J. Zhong, Physicochemical properties and formation mechanism of whey protein isolate-sodium alginate complexes: Experimental and computational study, Food Hydrocoll. 131 (2022) 107786. https://doi.org/10.1016/J.FOODHYD.2022.107786.

[55] Z. Wang, J. Chandrapala, T. Truong, A. Farahnaky, Multicomponent Oleogels Prepared with High- and Low-Molecular-Weight Oleogelators: Ethylcellulose and Waxes, Foods 12 (2023). https://doi.org/10.3390/foods12163093.

[56] M. Davidovich-Pinhas, S. Barbut, A.G. Marangoni, The gelation of oil using ethyl cellulose, Carbohydr. Polym. 117 (2015) 869–878. https://doi.org/10.1016/j.carbpol.2014.10.035.

[57] H.Y. Wu, C.B. Sun, N. Liu, Effects of different cryoprotectants on microemulsion freeze-drying, Innov. Food Sci. Emerg. Technol. 54 (2019) 28–33. https://doi.org/10.1016/j.ifset.2018.12.007.

[58] T. Yilmaz, L. Maldonado, H. Turasan, J. Kokini, Thermodynamic mechanism of particulation of sodium alginate and chitosan polyelectrolyte complexes as a function of charge ratio and order of addition, J. Food Eng. 254 (2019) 42–50. https://doi.org/10.1016/j.jfoodeng.2019.03.002.

[59] W. Xu, Y. Tang, Y. Yang, G. Wang, S. Zhou, Establishment of a stable complex formed from whey protein isolate and chitosan and its stability under environmental stresses, Int. J. Biol. Macromol. 165 (2020) 2823–2833. https://doi.org/10.1016/j.ijbiomac.2020.10.130.

[60] L.M. Gradinaru, M. Barbalata-Mandru, A.A. Enache, C.M. Rimbu, G.I. Badea, M. Aflori, Chitosan Membranes Containing Plant Extracts: Preparation, Characterization and Antimicrobial Properties, Int. J. Mol. Sci. 24 (2023). https://doi.org/10.3390/ijms24108673.

[61] F. Wang, C. Xie, H. Tang, W. Hao, J. Wu, Y. Sun, J. Sun, Y. Liu, L. Jiang, Development, characterization and application of intelligent/active packaging of chitosan/chitin nanofibers films containing eggplant anthocyanins, Food Hydrocoll. 139 (2023). https://doi.org/10.1016/j.foodhyd.2023.108496.