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Formulation development and physicochemical evaluation of hemp seed oil cleansing balms. The impact of activated charcoal on stability and rinsability

Opracowanie formulacji i ocena fizykochemiczna balsamów oczyszczających z olejem z nasion konopi. Wpływ węgla aktywnego na stabilność i spłukiwalność

ABSTRACT

Introduction: Hemp seed oil has attracted growing interest in cosmetic sciences due to its content of bioactive compounds with antioxidant and anti-inflammatory properties. As an ingredient in cleansing balms, it helps to liquefy makeup and impurities during massage.

Aim: The study aimed to develop and characterize makeup-remover balm formulations containing hemp seed oil.

Materials and methods: Formulations were evaluated for visual homogeneity, texture consistency, and high physical stability, with no phase separation or wax crystallization. Later, they were characterized by viscosity and density measurements, microbiological testing, stability assessment, and *in vivo* pilot patch testing.

Results: The formulation containing activated charcoal, sunflower oil, olive oil, and sweet almond oil showed the best overall performance. Microbial testing confirmed compliance with safety limits, including assessment against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Candida albicans*, as well as total aerobic mesophiles, mold, and yeast counts. The product remained physically and microbiologically stable.

Conclusions: Hemp seed oil shows potential as an ingredient in makeup removal balms. Research is underway to expand its use in the cosmetics industry.

Keywords: balm, hemp seed oil, patch test, skin cleansing.

STRESZCZENIE

Wprowadzenie: Olej z nasion konopi budzi coraz większe zainteresowanie w kosmetologii ze względu na zawartość związków bioaktywnych o działaniu przeciwutleniającym i przeciwzapalnym. Jako składnik balsamów oczyszczających wspomaga upłynnianie podczas masażu makijażu i zanieczyszczeń.

Cel: Celem badania było opracowanie oraz ocena właściwości receptur balsamów do demakijażu na bazie oleju z nasion konopi.

Materiały i metody: Preparaty oceniono wizualnie pod kątem jednorodności, konsystencji oraz stabilności fizycznej, wykluczając rozwarstwianie faz czy krystalizację wosku. Następnie scharakteryzowano je poprzez pomiary lepkości i gęstości, badania mikrobiologiczne, ocenę stabilności oraz pilotażowe testy płatkowe *in vivo*.

Wyniki: Najwyższą skutecznością charakteryzowała się receptura zawierająca węgiel aktywowany oraz oleje: słonecznikowy, z oliwek i ze słodkich migdałów. Badania mikrobiologiczne potwierdziły zgodność z normami bezpieczeństwa – wykluczono obecność *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* oraz *Candida albicans*, a ogólna liczba bakterii tlenowych mezofilnych, pleśni i drożdży mieściła się w dopuszczalnych limitach. Produkt zachował stabilność fizyczną i mikrobiologiczną.

Wnioski: Olej z nasion konopi wykazuje potencjał jako składnik balsamów do demakijażu. Prowadzone są badania w zakresie jego szerszego zastosowania w przemyśle kosmetycznym.

Słowa kluczowe: balsam, demakijaż, olej z nasion konopi, test płatkowy, oczyszczanie skóry.

INTRODUCTION

The first and most essential step in any skincare regimen is cleansing, and for this purpose, a wide variety of cleansing products have been developed, tailored to consumer needs based on skin type, age, gender, and underlying dermatological conditions [1, 2]. Cleansers should effectively remove impurities from the skin while preserving its natural moisture balance. They must be easy to apply and rinse off, leaving no residue or occlusive film. An ideal formulation should cleanse without causing dryness, tightness, or greasiness. During use, impurities should be dissolved and then removed by rinsing or wiping. There is a broad range of cleanser formulations, including soaps, syndets, foams, gels, oils, creams, milks, micellar waters, lotions, and cleansing balms [1, 3].

Cleansing balms are semi-solid, anhydrous systems that are particularly effective in removing heavy or waterproof makeup, surgical cosmetics, and sunscreens. These formulations resemble cold cream cleansers but they have a petroleum jelly-like consistency at room temperature. Cleansing balms with a melting point close to skin temperature soften upon contact with a skin, facilitating easy application and enhanced spreadability [1, 4]. Upon application, the balm melts into an oil-like layer that may require a second cleansing step, typically with water and a mild cleanser, especially for individuals with oily skin [1]. They are primarily composed of natural or herbal oils structured with waxes or butters, yielding a resilient, ointment-like consistency at room temperature [1, 4]. Owing to their anhydrous nature, cleansing balms have a lower risk of microbial contamination than hydrous ones, and are generally well tolerated across different skin types [4, 5].

Cleansing balms generally preserve the skin's moisture barrier and maintain physiological pH, thereby minimizing dryness and reducing the risk of irritation. They are particularly beneficial in dry or cold climates and for individuals with skin conditions such as eczema. These formulations are well-suited for individuals with dry or sensitive skin due to their ability to hydrate, calm irritation, and support barrier function. They may also have some disadvantages: their thick consistency can leave an oily residue, which may be unappealing to some users; and if the balms are not rinsed off properly, they may contribute to comedones [4]. Additionally, certain ingredients in cleanser formulations may cause irritation in individuals with sensitive skin [6].

Typical balm formulations include a lipid-rich base, structuring agents, low-level emulsifiers, and in some cases, active ingredients [1, 4]. Triglyceride oils and fatty acid esters form the continuous lipid matrix in cleansing balm formulations, acting as solvents for lipophilic active ingredients and modulators of skin permeability. Fatty acid esters serve as both solvents and penetration enhancers by creating microdomains of increased lipid mobility within the formulation [1, 7]. Solid lipids contribute to the structural integrity and film-forming properties of the balm. These ingredients impart occlusive properties, thereby minimizing transepidermal water loss and enhancing the activity of humectants. They also act as soothing and softening agents [8]. Semi-solid butters, such as shea butter, can be added to formulations to improve skin smoothness and texture [1, 9]. Surfactants and emulsifiers are essential components of cleansing balm formulations. Upon contact with water during rinsing, the balm forms a water-in-oil emulsion that encapsulates impurities within micellar structures, facilitating their efficient removal [8, 10]. When surfactants and fatty amphiphiles are combined into mixed emulsifiers or emulsifying waxes, they enable emulsion formation, enhance shelf-life stability, and modulate rheological properties through interactions with the aqueous phase and other excipients [11]. Functional actives, such as antioxidants (e.g., tocopherol) and clay minerals (e.g., kaolin), could be dispersed throughout the formulation to deliver targeted skincare benefits. Moisturizing agents such as glycerin and propylene glycol are also commonly added to enhance skin hydration [8].

In recent years, consumer demand for natural and plant-based ingredients in skincare and beauty products has been steadily increasing. The incorporation of natural materials in cosmetics is becoming more and more popular, as these ingredients offer diverse benefits depending on their origin and help to reduce exposure to synthetic chemicals [12]. One such natural ingredient gaining attention in the health and beauty industry is hemp seed oil obtained from *Cannabis sativa* L. [13].

The cosmetic industry has recently shown growing interest in hemp seed oil [14]. It is a potent anti-inflammatory agent that supports cellular regeneration, making it effective in reducing skin irritation, while maintaining skin hydration and nourishment [15, 16]. Hemp seed oil, with its distinctive essential fatty acid profile and optimal omega-6 to omega-3 ratio (1:3), plays a significant role in supporting skin health and restoring barrier function [15]. Moreover, it also possesses anti-aging properties, helping to prevent fine lines, wrinkles, and other signs of aging. The antioxidants in hemp seed oil promote cell regeneration and support faster skin healing [17]. The oil also stimulates collagen production, essential for maintaining smooth, elastic skin. Its use may help manage dermatological conditions such as atopic dermatitis and acne [18, 19]. Additionally, vitamin E and carotene support the maintenance of healthy skin [20], while the oil's fatty acids help regulate sebum production and reduce inflammation associated with acne. Carotenoids also protect against UV light can combat free radicals [14]. Hemp seed oil also has antimicrobial activity [15].

While hemp seed oil offers many advantages, it presents formulation challenges, mainly due to its high polyunsaturated fatty acid content, which makes it prone to oxidation. Studies showed that the rate of lipid oxidation is associated with temperature exponentially; therefore, processing parameters in preparation of balm formulations are critical, and may result in a change in loss of hemp seed oil bioactivity, skin irritation problems, and stability issues in the formulation, and may reduce the shelf life of the product [21-23].

AIM OF THE STUDY

Owing to the superior properties of hemp seed oil and the formulation challenges associated with its incorporation into cosmetic products, this study aimed to develop and optimize a makeup removal balm containing hemp seed oil and to evaluate its physicochemical characteristics, microbiological quality, and stability to assess its potential as a cosmetic cleansing product.

MATERIALS AND METHODS

Materials

Various cosmetic-grade ingredients were used in the formulation development studies for the cleansing balms. *Cannabis sativa* seed oil obtained from, Cansızzade Bitkisel Yağlar (Türkiye), contained in its composition linoleic acid (C18:2) at 55.13%, linolenic acid (C18:3) at 22.50%, oleic acid (C18:1) at 12.20%, palmitic acid (C16:0) at 7.01%, stearic acid (C18:0) at 2.13%, and arachidonic acid (C20:4) at 1.03%. Ricinus *communis* seed oil (castor oil), *Butyrospermum parkii* (shea) butter, *Rhus succedanea* fruit wax, *Prunus amygdalus dulcis* (sweet almond) oil, *Cocos nucifera* (coconut) oil, *Euphorbia cerifera* (candelilla) wax, *Helianthus annuus* (sunflower) seed oil, *Olea europaea* (olive) fruit oil, polysorbate 80, tocopherol, phenoxyethanol, caprylyl glycol, glyceryl stearate, cera alba (beeswax), stearic acid, hydrogenated polyisobutene, ethylhexyl palmitate, vaseline (petrolatum), polyamide-8, geraniol, and citronellol were sourced from Hammaddesepeti (Türkiye). Cetyl alcohol, caprylic/capric triglyceride, and hydrogenated castor oil were obtained from BASF (Germany). Isopropyl myristate, tetradecyl myristate, ethylhexyl stearate, ceteareth-25, kaolin, and emulsifying wax NF (cetearyl alcohol and polysorbate 60) were acquired from Croda (Germany). PEG-20 glyceryl triisostearate, Crodamol™ IPIS (isopropyl isostearate), were obtained from Croda (England). Glycereth-2 cocoate was acquired from Levenol H&B (Spain). Polyamide-3 (TEGO®) was sourced from Evonik (Germany). Sorbitan olivate and cetearyl olivate (OliveM 1000) were supplied by Univar Solutions (USA). Charcoal powder was provided by CarboTech (Germany).

Devices and instruments

High-speed homogenization was carried out using the WiseTis HG-15D (South Korea) and IKA T25 Easy Clean (Germany) homogenizers. Weighing procedures were performed with an Ohaus Explorer Analytical Plus analytical balance (USA). Temperature-controlled experiments were conducted using a WiseBath water bath (Japan). Viscosity measurements were performed using a Brookfield viscometer (Canada), while optical observations of formulations were evaluated using a Leica Zoom 2000 stereo zoom microscope (Germany). Measurements of pH were recorded with a Hanna Edge pH meter (USA). Stirring and controlled heating during the preparation process were facilitated using a Heidolph magnetic stirrer with a heating plate (Germany). A Kimble Chase picometer (USA) was employed for micro-volume measurements where required.

Selection of formulation ingredients

The ingredients used in cleansing balm formulations and their respective functions are presented in tab. 1. In summary, oils were incorporated in formulations to dissolve sunscreen, makeup, and sebum while simultaneously nourish the skin. Waxes gave the balm solid consistency and helped regulate the melting point. Emulsifiers and surfactants enabled the oil phase to be rinsed off with water, ensuring effective removal of dirt and makeup residues. Charcoal powder helped purify the skin, while kaolin absorbed excess sebum and mattified the skin [24].

General procedure for preparation of the cleanser balm formulations

During formulation studies, the ingredients were divided into two phases: Phase A consisted of ingredients that are solid at room temperature but melt with heat and do not decompose, such as waxes, esters, and emulsifiers. Phase A was melted in a water bath at 70-75°C until a clear, homogeneous phase was obtained. Gentle magnetic stirring (approximately 200 rpm) was maintained until all ingredients were fully liquefied and homogeneous. Prolonged exposure to high heat was avoided to reduce the risk of oxidation.

In Phase B, temperature-sensitive compounds such as tocopherol, fragrances, OliveM 1000, glycereth-2 cocoate, and certain oils, including hemp seed oil, were heated in a separate vessel to 40°C. Phase B was then gradually added to Phase A under constant stirring with a homogenizer at 300-500 rpm. Mixing was continued until a uniform balm matrix was formed. The mixture was subsequently cooled slowly under agitation to prevent phase separation. During the studies, 10 formulations were prepared and labelled as F1-F10. The ingredients used for formulations and the their composition (weight-by-weight percent, w/w%) are given in tab. 2.

Tab. 1. Ingredients used in cleansing balm formulation and their functions. **Source:** [24, 25].

Main Group	Ingredient	Function
Emollients & Oils		
	Hemp Seed Oil	Emollient, moisturizes skin, dissolves makeup and sebum
	Butyrospermum Parkii (Shea) Butter	Natural moisturizer, protects skin barrier
	Petrolatum	Occlusive, emollient
	Castor Oil	Emollient, oil-based cleanser
	Sweet Almond Oil	Light emollient
	Sunflower Seed Oil	Non-comedogenic oil, supports skin barrier
	Olive Oil	Emollient, oil-based cleanser
	Isopropyl Myristate	Emollient, improves spreadability
	Ethylhexyl Stearate	Emollient, enhances spreadability
	Ethylhexyl Palmitate	Emollient and solvent
	Hydrogenated Polyisobutene	Emollient
	Crodamol™ IPIS	Emollient
	Tetradecyl Myristate	Emollient
Emulsifiers & Surfactants		
	Emulsifying Wax	Emulsifier
	Glyceryl Stearate	Emulsifier
	Polysorbate 80	Solubilizer, surfactant
	Polyglyceryl-3 Diisostearate	W/O Emulsifier
	PEG-20 Glyceryl Triisostearate	Surfactant, enhances cleansing
	Glycereth-2 Cocoate	Mild surfactant and solubilizer
	OliveM 1000	Emulsifier
Thickeners / Film Formers		
	Cetyl Alcohol	Fatty alcohol, emollient, emulsifier and thickener
	Kaolin Clay	Oil absorber
	Charcoal Powder	Adsorbs impurities
	Rhus Succedanea Fruit Wax	Thickener
	Beeswax	Thickener
	Stearic Acid	Thickener, emulsifier
	Candelilla Wax	Thickener
	Hydrogenated Castor Oil	Thickener and stabilizer
	Polyamide-3 / Polyamide-8	Film formers
Preservatives & Antioxidants		
	Phenoxyethanol	Broad-spectrum preservative
	Caprylyl Glycol	Preservative booster and humectant
	Caprylic Acid	Skin-conditioning, mild antimicrobial agent
	Tocopherol (Vitamin E)	Antioxidant
Fragrance Components		
	Geraniol	Fragrance compound
	Citronellol	Fragrance compound

Tab. 2. The ingredients used in each formulation (w/w%). **Source**: Own elaboration.

Ingredients (w/w%)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Hemp Seed Oil	38	20	2	2	1	6	6	7	5	5
Sunflower Oil					**8**	**65**			**5**	**5**
Olive Oil									5	5
Tocopherol	1	1			0.5					
Sweet Almond Oil					10				1	1
Phenoxyethanol+Caprylyl Glycol			1		1	1	1	1		
Hydrogenated Polyisobutene								43		
Isopropyl Myristate	15				10		20			
Castor Oil	10									
Emulsifying wax	7				19	20	18			
Kaolin Clay	6									
Polysorbate-80	5									
Glycereth-2 Cocoate			20							
Polyamide-8			6							
Polyglyceryl-3 Diisostearate			6							
Shea Butter		7	10	5	5			29	10	10
Rhus Succedanea Wax			10	5						
Tetradecyl myristate			2							
Ethylhexyl Stearate			43	37						
Stearic acid									3	3
Glyceryl stearate									8	8
Cera Alba (Beeswax)					10	8		20	10	10
Caprylic/Capric Triglyceride		5			20		55		42	40
Cetearth-25									10	10
Geraniol									0.1	0.1
Citronellol									0.1	0.1
Charcoal Powder										2
Polyamide-3				10						
PEG-20 Glyceryl Triisostearate				20						
Polyglyceryl-3-Diisostearate		2		3	5					
Ethylhexyl palmitate				5						
Crodamol™ IPIS				10						
Castor Oil				3						
Parfum					0.5				0.8	0.8
Cetyl Alcohol	18									
White Petrolatum		60								
Candelilla Wax		5								
OliveM 1000					10					

Characterization studies of the formulations

Visual analyses

All formulations were visually analyzed for color, odor, texture, phase separation, wax crystallization, and homogeneity. To account for potential changes over time, visual analyses were also carried out during the physical and microbiological stability studies.

Microscopic examinations

Microscopic and morphologic examinations of the balms were carried out using a Leica 2000 Stereo microscope (Leica Microsystems, Wetzlar, Germany) at 1.5x magnification. The microscope investigations were performed to assess phase separation, residues, and the morphology of the formulations. During studies, each formulation was applied to a glass slide using a stainless-steel spatula. Samples were gently spread into a uniform film (~2 mm thickness) to ensure comparable surface area across all formulations. All experiments were performed under controlled ambient conditions.

Preliminary rinsability screening of formulations

A preliminary rinsability screening test has been developed by modifying an existing test from the literature [26]. This test was performed to visually evaluate residual formulation deposits on a glass slide after rinsing. Although glass slides do not replicate the complexity of human skin, the test provides preliminary insight into the film-forming properties and rinsability of the formulations. In the tests, all ten formulations were applied onto the center of glass slides (selected as non-porous substrates) in equal amounts using a spatula. To assess the rinsability of the formulations, each prepared slide was exposed to running tap water for 3 seconds, during which the surface was gently massaged in circular motions with a gloved fingertip. To evaluate how effectively each formulation was removed, the extent of residual product on the non-porous substrate and surface shine or gloss were recorded after rinsing. The slides were visually inspected for any residual oil films, providing insight into the formulation's rinsability and presence of potential residue. Images of the formulations on the glass slides were taken when the formulations were applied and after the balm formulations had risen with water.

Density measurements

The density of a cosmetic formulation was measured by using a calibrated glass pycnometer. After calibration, the pycnometer was then filled with the test sample to the calibration mark. The known density of water at 20°C was used for reference. Before sample measurement, air bubbles in the cosmetic formulation were removed using a gentle vacuum or sonication to ensure accuracy [27].

Viscosity measurements

The viscosity of formulations F9 and F10 were measured using a Brookfield DVT3 rotational viscometer equipped with an RV-06 spindle operated at 5 rpm. Prior to analysis, samples were homogenized, degassed, and allowed to equilibrate at 20°C ± 1°C. Measurements were performed in appropriate sample containers to minimize air entrapment, with the spindle immersed to the specified depth. Viscosity values were recorded after stabilization of the readings. All measurements were conducted at ambient conditions and performed in triplicate [28].

Microbiological tests

Microbiological tests of the formulation F10 were conducted at Bilim Laboratories in İstanbul, Türkiye. The tests were carried out at the company's Cosmetic and Industrial Product Analysis Laboratory. During studies, three samples of the formulation F10 in 50 g plastic packages were analyzed. Microbiological neutralization and inoculation procedures were conducted. All tests were performed in accordance with the ISO 17516:2014 standard for microbiological quality of cosmetic products.

To evaluate the formulation's antimicrobial activity, neutralization studies were performed using relevant test microorganisms to verify the suitability of the analytical method. For each organism, an initial suspension was prepared by diluting the sample at a 1:9 ratio (sample to neutralizing diluent). Eugon LT 100 Broth was utilized uniformly as the neutralizing diluent across all tests. The suspensions were then cultured on selective media specific to each microorganism: *Pseudomonas aeruginosa* on Cetrimide Agar, *Escherichia coli* on MacConkey Agar, *Staphylococcus aureus* on Baird Parker Agar, *Candida albicans* on Sabouraud Dextrose Chloramphenicol Agar, and aerobic mesophilic bacteria on Tryptic Soy Agar using the cast plate method. For molds and yeasts, inoculation was performed on Potato Dextrose Agar using the smear plate method. The effectiveness of the neutralization procedure was confirmed for each organism, validating the method's reliability for determining the antimicrobial efficacy of the test sample. As per microbial limit criteria, these pathogenic microorganisms must be absent in the product (absent in 1 g/1 mL).

Physical and microbiological stability tests

Stability testing was performed to assess the physical, microbiological, and sensory stability of a cosmetic product's formulation over a period of three months under various temperature conditions. These tests aimed to ensure that the product remains effective, safe, and aesthetically acceptable throughout both storage and usage conditions [29]. Stability tests of the products were conducted at Bilim Laboratories in İstanbul, Türkiye. The tests were carried out at the company's Cosmetic and Industrial Product Analysis Laboratory. Studies

were carried out only with the F10 samples, and during the studies, three samples of the hemp-containing make-up removal balm in 50 g plastic packages were analyzed. The parameters investigated during the stability test and the methods used during testing are given in tab. 3.

Tab. 3. The parameters investigated and the methods used for stability testing (CFU: colony-forming unit). **Source:** Own elaboration.

Parameter	Unit	Method
Total aerobic count	CFU/g	TS EN ISO 21149
Yeast and mould	CFU/g	TS EN ISO 16212
Candida albicans	CFU/g	TS EN ISO 18416
Pseudomonas aeruginosa	CFU/g	TS EN ISO 22717
Escherichia coli	CFU/g	TS EN ISO 21150
Staphylococcus aureus	CFU/g	TS EN ISO 22718
Colour	-	Visual inspection
Smell	-	Visual inspection
pH	-	In-house Method – P13-FB-T148
Density	g/cm³	In-house Method – P13-FB-T230
Packaging test	-	Inhouse Method

Stability tests were conducted to investigate the permanency of balm formulations under different conditions. These tests were performed at room temperature – daylight conditions, room temperature – dark conditions, 4°C ± 2°C, and 40°C ± 2°C/ 75% RH ± 5% RH for 90 days. The samples were analyzed on the 1st, 28th, and 90th days. At the end of 90 days, the stability testing results were evaluated against the predefined acceptance criteria.

Packing of the balm formulations

Before stability testing, balm formulations were filled into 50 g-capacity, food-grade acrylic jars. In this study, acrylic containers were chosen for their durability, lightweight nature, clarity, and aesthetic appeal. Acrylic packaging is known for its high resistance to breakage, strong barrier properties, and ability to present products attractively to consumers [30]. Immediately after blending, each formulation was maintained at ~40°C to preserve fluidity and then transferred aseptically into pre-sterilized jars using a sterile spatula. Filled jars were conditioned at ambient laboratory conditions (22°C ± 2°C, 45% ± 5% RH) for 24 h to complete solidification.

Pilot patch test for dermatological evaluation

A dermatological pilot patch test was conducted at SKINLAB P.S.A. in Kraków, Poland, to evaluate the preliminary potential irritant and sensitizing effects of the hemp seed oil-containing make-up remover balm formulation F10 in 50 g plastic packages. The pilot test was conducted between April 15 and April 24, 2025, on 10 healthy volunteers with standard skin types. The final evaluation was completed on May 20, 2025. The original laboratory report has been included in the supplementary materials.

The study was initiated upon the Principal Investigator's submission of an official order form and the corresponding test samples. The Principal was responsible for ensuring that the tested product complied with the declared composition and microbiological purity.

The patch test was conducted in accordance with applicable regulatory and ethical requirements, including Regulation of the European Parliament and Council Regulation on cosmetic products (EC) No 1223/2009 of November 30, 2009, the Cosmetics Europe The Personal Care Association Guidelines "Product Test Guidelines for the Assessment of Human Skin Compatibility 1997", the WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects (1964-2013), the Act on Cosmetic Products, October 4, 2018, Journal of Laws No. 2018 item 2227. The study also followed SKINLAB P.S.A.: internal procedures and instructions (PO-08 Research Implementation, I02/PO-08 Dermatological test – patch test, I04/PO-08 Scheme for assessing skin reactions and product classification).

Volunteer recruitment and qualification were performed in accordance with current European and Polish legislation, Cosmetics Europe guidelines, the Declaration of Helsinki, and SKINLAB P.S.A. procedures (PO-08 Research Implementation and I01/PO-08 Volunteers Qualification for the Study). Exclusion criteria included the use of antibiotics, pregnancy or breastfeeding, ongoing chemotherapy or radiotherapy, fever, active infections such as the common cold, the use of anti-allergic medications or ointments, the presence of skin lesions, and age below 18 years. All volunteers selected for the study met the inclusion criteria, provided informed consent to participate, and were informed of the study's purpose, its conduct, and possible side effects. During the entire study, the volunteers were under the constant care of a dermatologist.

The test was performed in accordance with the research procedure of SKINLAB P.S.A. (PO-08 Research implementation) under the supervision of a dermatologist. The study design was based on the Jadassohn-Bloch patch test, as modified by Rudzki et al. [31]. The test consisted of a single application of the product to a selected area of the skin, followed by observation of the skin at intervals. Skin reactions were evaluated at specific intervals post-application using a 0-4 scale for grading dermal responses. The average irritation index for the tested product was calculated by dividing the sum of scores to the number of volunteers. The sum of the scores for erythema and edema are used for the calculation. All assessments, including volunteer qualification, sample application, and result interpretation, were conducted on-site at SKINLAB P.S.A. in Kraków.

RESULTS

Formulation studies

In the study, 10 formulations with various ingredients were first prepared, and afterward, characterized as described in the Materials and Methods section. The ingredients used for formulations and the composition of formulations (weight-by-weight percent, w/w%) are given in tab. 2.

Visual analyses

All formulations were visually analyzed for color, odor, texture, phase separation, wax crystallization, and homogeneity. Visual analyses of all the formulations are reported in tab. 4, and images of the formulations are given in fig. 1. In terms of appearance, the formulations exhibited distinct color characteristics. F1 was greyish beige, while F2 displayed a light cream color. The phase-separated formulations (F3-F5) exhibited yellow-based hues ranging from pale yellow to yellow-green. Formulations F6-F9 showed neutral, light-toned colors varying from beige to white. In contrast, F10 exhibited a jet-black appearance.

With respect to texture, visual formulation stability after production, and formulation homogeneity, formulations F3, F4, and F5 showed visible phase instability within 24-48 hours post-production, including surface oiling-out, dull texture, and uneven consistency. In terms of texture, F4 also did not align with the intended formulation goal. Instead of achieving a balm-like texture, the formulation displayed a fluid consistency, which was not suitable for the targeted application properties. Formulations F6, F7, and F8 did not exhibit phase separation; however, they showed a non-homogeneous texture and exhibited wax crystallization, suggesting non-uniform formulation and incomplete oil dispersion. The results revealed that the formulations F1, F2,

Tab. 4. Visual analysis of the balm formulations. **Source:** Own elaboration.

Formulation code	Color	Odor	Texture	Phase separation and/or wax crystallization	Homogeneity
F1	Greyish-beige, opaque	Neutral	Butter	No	Homogenous
F2	Light cream, semi-opaque	Neutral	Cream	No	Homogenous
F3	Light beige, semi-translucent	Neutral	Balm	Phase separation, wax crystallization	Non-homogenous
F4	Pale yellow, translucent	Neutral	Fluid/ Lotion-like	Phase separation	Non-homogenous
F5	Yellow-green, semi-opaque	Neutral	Balm	Phase separation, wax crystallization	Non-homogenous
F6	Beige, semi-opaque	Neutral	Balm	Wax crystallization	Non-homogenous
F7	Off-white, opaque	Neutral	Balm	Wax crystallization	Non-homogenous
F8	White, opaque	Neutral	Balm	Wax crystallization	Non-homogenous
F9	White, opaque	Neutral	Balm	No	Homogenous
F10	Jet black, opaque	Neutral	Balm	No	Homogenous

Fig. 1. Images of the balm formulations. **Source:** Own elaboration.

F9, and F10 maintained physical stability over the evaluation period, exhibiting no phase separation, odor alteration, or surface blooming. In terms of texture, formulations F1 and F2 were not found suitable for the targeted application. The F1 formulation, which had a butter-like consistency, was identified as the most difficult to spread. Formulation F2 had a cream-like consistency.

Microscopy images

Microscopy images of the balm formulations were taken using a Leica Zoom 2000 stereo zoom microscope (Germany) at 1.5x magnification, and the results are reported in fig. 2.

According to microscopy examination, F1 exhibited a heterogeneous semi-solid structure with localized regions of partial melting due to the light source during microscope illumination. F2 appeared as a continuous, translucent, oil-rich film with a uniform texture. F3 exhibited clear phase separation, with contiguous translucent lipid regions. F4 manifested as a non-homogeneous, non-crystalline fluid structure. F5 showed semi-continuous lipid crystal structures with denser, angular aggregates at the periphery transitioning into a smoother, more homogeneous central phase. F6 revealed a compact structure with lipid crystals. F7 displayed a heterogeneous semi-solid structure. F8 demonstrated satisfactory spreadability on the lam surface, however, it exhibited a non-homogeneous structure under microscopic examination. F9 had a semi-solid formulation. F10 was characterized by a dense structure of particulate aggregates, which was attributable to activated charcoal, embedded within a continuous lipid phase.

Preliminary rinsability screening of formulations

A qualitative rinsability screening was performed on a non-porous glass substrate to compare the tendency of formulations to emulsify and detach upon brief water exposure. Images of the balm formulations were taken before and after testing, and the results are given in fig. 3.

F1 left a virtually medium amount of residue or oily shine on the glass slide after washing, despite its butter-like texture. F2, on the other hand, left excessive oiliness and a jelly-like texture, which formed a transparent film after rinsing. It

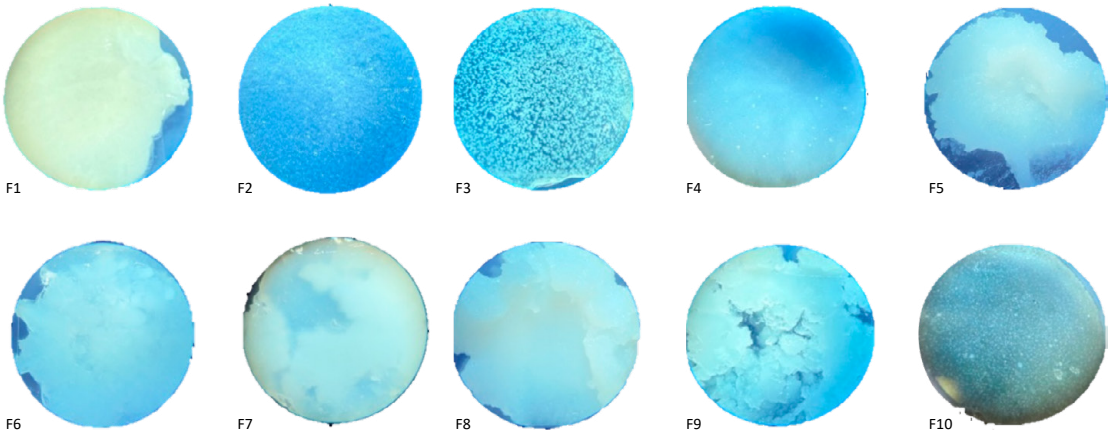

Fig. 2. Images of the formulations using a Leica Zoom 2000 stereo zoom microscope at 1.5x magnification. **Source:** Own elaboration.

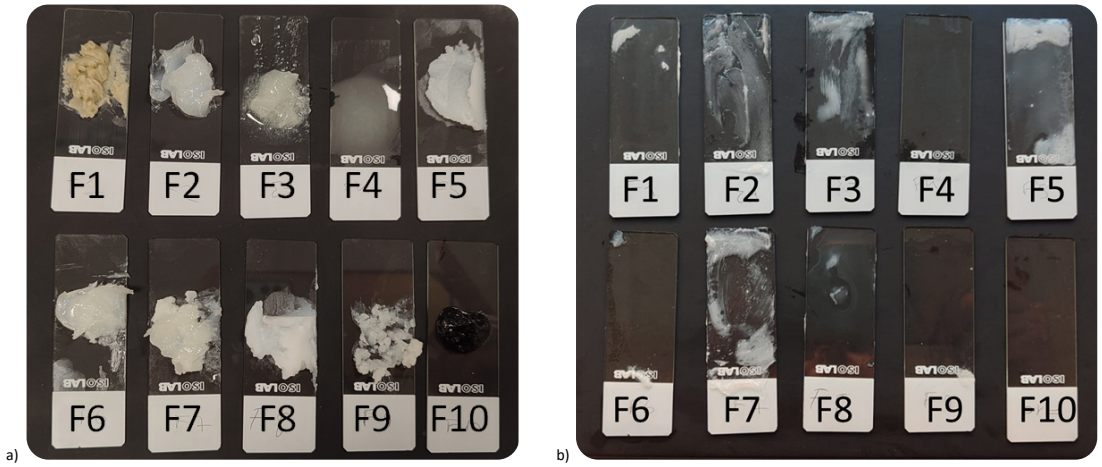

Fig. 3. Images of formulations on glass slides before (a) and just after (b) preliminary rinsability screening. Glass slides were selected as a non-porous substrate during studies. **Source:** Own elaboration.

is observed that both formulations F3 and F5 had a high sediment content; F3 exhibited phase separation, rendering it unsuitable; and its cleansing performance fall below expectations For F5, it was observed that the formulation spread well on the glass slide, however localized residue buildup was observed; which further adjustment of emulsifier balance might reduce leftover film. Although F4 was overly fluid for practical use and had no balm texture, it was found to have very good rinsability. Formulations F6 and F7 were found to be similar to F2 in terms of appearance; however, they mildly outperform F2 due to slightly lower residue on slides, which may indicate a need for surfactant optimization. F8 mirrored F5's creaminess with consistent residue formation. F9 and F10 had similar formulations, except that F10 contains activated charcoal. It is observed that the addition of activated charcoal to F10 enhances cleansing efficiency compared to F9 by preventing residual particles.

Density and viscosity measurements

Density and viscosity measurements were carried only with formulations F9 and F10, as these are the formulations that maintained physical stability and exhibit balm texture. Due to observable phase separation (F3-F5) and non-homogeneity (F3-F8), density and viscosity measurements were not performed for all formulations. Reliable determination of these parameters required a homogeneous system; these samples might yield non-representative, misleading results by reflecting only the properties of individual phases rather than the bulk formulation. Formulations F1 and F2 were also eliminated because they exhibit butter and cream textures rather than a balm texture, respectively. According to the results, formulations F9 and F10 both exhibited a density of 0.90 g/cm^3.

For the viscosity measurements of the balm formulations, the formulations F1-F8 were not further evaluated, similar to the density measurements. The viscosity of F9 was 103.333 ± 6.236, while F10 was 115.000 ± 7.071.

Microbiological tests

The assessment protocol included challenge tests against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans*, as well as determinations of total aerobic mesophiles (Total Aerobic Mesophilic Count, TAMC) and mold and yeast counts (Total Yeast and Mold Count, TYMC).

In order to assess the microbiological safety of the developed cosmetic formulations, a series of microbiological analyses were conducted. These analyses aimed to determine both the total microbial load and the presence of specific pathogenic microorganisms that might pose a risk to human health. Only the formulation F10 was advanced to microbiological evaluation. Other formulations were not subjected to microbial testing since F1-F8 failed earlier performance criteria, and F9 remained under further optimization.

The results for F10 are given in tab. 5. Aerobic mesophilic bacteria and mold/yeast counts were both below the limit of quantification (<10 CFU/g), within the regulatory threshold of 1×10^3 CFU/g. No colonies of *Escherichia coli, Pseudomonas aeruginosa, Candida albicans*, or *Staphylococcus aureus* were detected, confirming the absence of these pathogens in the tested samples [32].

Physical and microbiological stability tests

Stability tests were conducted to investigate the stability of balm formulations under different conditions. These tests were conducted at room temperature – daylight conditions, room temperature – dark conditions, 4°C ± 2°C and 40°C ± 2°C/75% RH ± 5% RH for 90 days (Tab. 6).

Stability testing at all conditions for 90 days showed that total aerobic count (CFU/g), as well as yeast and mould (CFU/g) were found to be <10. *Candida albicans, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* were not detected within samples through the studies up to 90 days. Changes in color and smell were assessed by comparison with the initial formulation prior to testing. F10 remained visually stable, with no change in texture, color, or odor. No significant change in the pH was detected. During the studies, the sample passed the packing test; no cracks or leaks were detected in the packing material. The stability testing results demonstrated compliance across all measured parameters.

Tab. 5. Microbial quality assessment results of the cleansing balm formulation F10. **Source:** own elaboration.

Parameter	Result	Limit*	Method	Compliance
Aerobic mesophilic bacteria count	< 10 CFU/g	< 1 × 10^3 CFU/g	TS EN ISO 21149	Compliant
Mold and yeast count	< 10 CFU/g	< 1 × 10^3 CFU/g	TS EN ISO 16212	Compliant
Escherichia coli detection	Not Detected	Should not be found	TS EN ISO 21150	Compliant
Pseudomonas aeruginosa detection	Not Detected	Should not be found	TS EN ISO 22717	Compliant
Candida albicans detection	Not Detected	Should not be found	TS EN ISO 18416	Compliant
Staphylococcus aureus detection	Not Detected	Should not be found	TS EN ISO 22718	Compliant

* Rinse-off products

Tab. 6. Stability test results at the end of 1st, 28th, and 90th days. No day designation is provided for values that remained constant throughout the study, only parameters exhibiting changes are reported. **Source**: Own elaboration.

Parameter	Room Temperature, Daylight Conditions	Room Temperature, Dark Conditions	4°C	40°C/75% RH
Total Aerobic Count (CFU/g)	<10	<10	<10	<10
Yeast and mould (CFU/g)	<10	<10	<10	<10
Candida albicans	Not detected	Not detected	Not detected	Not detected
Pseudomonas aeruginosa	Not detected	Not detected	Not detected	Not detected
Escherichia coli	Not detected	Not detected	Not detected	Not detected
Staphylococcus aureus	Not detected	Not detected	Not detected	Not detected
Colour	No change	No change	No change	No change
Smell	No change	No change	No change	No change
pH (1st, 28th, 90th day, respectively)	5.29, 5.24, 5.12	5.29, 5.19, 5.05	5.28, 5.16, 5.04	5.28, 5.05, 5.01
Density (g/cm^3)	0.90	0.90	0.90	0.90
Packaging test	No cracks or leaks detected	No cracks or leaks detected	No cracks or leaks detected	No cracks or leaks detected

Pilot patch test

The pilot patch test analyses for the formulation F10 are presented in tab. 7. The average irritation index was calculated using the sum of the scores for erythema and edema.

Tab. 7. Pilot Patch Test Results from The Test-Point Classification (F- female, M- male, N-normal, S-sensitive). **Source**: Own elaboration.

Volunteer Identification Number	Sex	Age	Skin Type	Results after 48 hours	
				Erythema	Edema/ Swelling
1	F	45	N	0	0
2	F	45	N	0	0
3	F	28	N	0	0
4	F	58	N	0	0
5	F	43	N	0	0
6	F	43	N	0	0
7	F	68	N	0	0
8	F	61	N	0	0
9	F	35	N	0	0
10	M	41	N	0	0

DISCUSSION

In this study, ten cleansing balm formulations containing hemp seed oil were developed. Hemp seed oil, incorporated as the main functional component in all balm formulations, offers unique therapeutic benefits due to its high concentration of polyunsaturated fatty acids, particularly linoleic acid (C18:2) and alpha-linolenic acid (C18:3) [33, 34]. These fatty acids are essential for maintaining epidermal barrier integrity, enhancing skin hydration, and supporting anti-inflammatory pathways [15]. The stability and performance of the balm formulations were found to depend strongly on formulation composition and the hemp seed oil ratio.

An ideal cleansing balm should exhibit a semi-solid consistency-retaining structural integrity at room temperature while readily softening upon contact with the skin to ensure ease of application. This property allows the product to melt at or slightly below body temperature (~32-35°C), facilitating effortless spreading and effective emulsification of impurities without the need for mechanical force. Such characteristics are essential for ensuring both product performance and consumer satisfaction. Therefore, visual analyses are very important for evaluating balm formulations [1, 4, 24].

Visual analyses revealed that the inclusion of hemp seed oil significantly influenced the formulation parameters across the tested samples. According to the results, the characteristic greyish-beige color of F1 was due to the high proportion of hemp seed oil (38%) used in the formulation.

Results revealed that some of the developed formulations exhibited suitable structural properties for use as hemp seed oil-based cleansing balms. Formulation F4's fluid/lotion-like consistency likely resulted from its high content of liquid ingredients, such as ethylhexyl stearate and PEG-20 glyceryl triisostearate.

Formulations F6, F7, and F8 did not exhibit phase separation; however, they showed a non-homogeneous texture and exhibited wax crystallization, suggesting non-uniform formulation and incomplete oil dispersion. This result could

indicate that higher-melting-point waxes and fatty alcohols may have solidified early in the cooling during the preparation process. Based on this data, the amounts of lower- and higher-melting-point ingredients were adjusted in the subsequent formulation studies (i.e., F9 and F10) to avoid this problem.

The F1 formulation, which had a butter-like consistency, was identified as the most difficult to spread. Furthermore, its high oil content resulted in a distinctly greasy balm. Formulation F2 had a cream-like consistency, likely due to its high hemp seed oil (20%) and white petrolatum (60%) content, as well as its lower wax content compared to other formulations. A closer examination of the formulations using microscopic analysis was evaluated in parallel with the visual observations.

Based on visual and microscopy analyses, the best formulations were selected as F9 and F10. These formulations did not exhibit phase separation, odor alteration, or surface blooming. They maintained a semi-solid yet spreadable texture, supported by emulsifier systems that allowed for even dispersion of the oil phase and effective interaction with the stratum corneum. The most spreadable balm was observed as the F10 formulation. This formulation was favored due to its ease of removal from the packaging with a spatula during studies and its observed ease of application. Formulations F9 and F10 shared almost identical base composition and exhibited the best performance in terms of optimized hemp seed oil concentration as 5%, as well as the presence of other ingredients that function to support skin integrity (sunflower seed oil), emollients (sweet almond oil, olive oil) and those to help dissolving make up (olive oil). Unlike F9, F10 was enriched with particulate activated charcoal to enhance its cleansing efficacy, resulting in its characteristic black color (Fig. 1). The inclusion of activated charcoal in F10 was intended to exploit its high adsorptive capacity, thereby facilitating the thorough removal of sebum, residual makeup, and environmental particulates from within the pores [24, 35]. Indeed, in the preliminary rinsability screening test, the incorporation of activated charcoal appeared to enhance the cleansing efficiency of F10 on the nonporous glass substrate.

Density affects the balm's sensory attributes and stability [36]. The density of the balm formulation ensures that the product feels substantial without being heavy and facilitates the stable dispersion of active ingredients and emulsifiers within the semi-solid matrix. Elevated densities are typically associated with a firmer texture and reduced malleability, which can hinder ease of application and initial spreadability.

According to the results, formulations F9 and F10 both exhibited a density of 0.90 g/cm³. Although specific literature values for the density of cleansing balm formulations are unavailable, the study included a comparative evaluation of cosmetic products in the Nigerian market, reporting specific gravity values of commercial balm products as 0.94 ± 0.06, and produced balms as ~1.07 ± 0.13 [37]. Since anhydrous cosmetic products do not contain water, and their density is primarily determined by the densities of constituent oils, waxes, and emollients. Based on this, the measured densities of F9 and F10 were considered reasonable based on the known densities of the raw materials used, (e.g., medium-chain triglycerides ~0.93-0.96 g/cm³, hemp seed oil ~0.92 g/cm³, shea butter ~0.85 to 0.94 g/cm³, beeswax ~0.96 [24, 38, 39]), and this compositional basis supports the plausibility of the measured density within the context of the formulation. The results suggested that F9 and F10 were likely to have the desired balance between solidity and rapid melting upon contact with skin, thereby promoting both user comfort, ease of application, and effective cleansing performance.

Viscosity influences the balm's texture, spreadability, and melting behavior upon skin contact [40, 41]. An optimal viscosity ensures that the balm maintains structural integrity at room temperature while allowing smooth application and transformation into an oil-like phase that effectively dissolves makeup and sebum [42]. From a user experience perspective, the rheological behavior of a cleansing balm should strike a balance between structural integrity and ease of application. The formulation should not exhibit excessive fluidity, which may result in uncontrolled spreading or dripping, nor should it be overly rigid, which could hinder its ability to be smoothly applied [4]. In literature, the viscosity range for lotions is in the range of 2,000-50,000 cP [43], while gels have a larger viscosity in the range of 10,000 to 100,000 cP [44]. Alchemy Ingredients reports a viscosity range of their collagen cleansing balm in the range of 80,000-130,000 cP (measured using a Brookfield DV-E viscometer at 10 RPM and 20°C) [45], and for their cleansing balm in the range of 90,000-140,000 cP (Spindle T-D (94) at 10 RPM) [46]. Compared to these values, the viscosities of F9 and F10 fall within the ranges of these balms. The charcoal-powder-added F10 showed a viscosity increase of roughly 11% relative to its unfilled counterpart (F9), likely due to the charcoal powder, which elevates viscosity.

The microbiological quality of F10 confirmed compliance with cosmetic microbiological requirements, indicating effective control of microbial contamination during formulation process. The absence of detectable aerobic mesophilic bacteria and mold/yeast growth suggests that the formulation provided an unfavorable environment for microbial proliferation, which may be associated to the anhydrous nature of the balm formulations.

Both physical and microbiological stability test results revealed that the formulation F10 was stable at all conditions studied [47].

Patch testing is a simple but important safety test to assess whether a cosmetic product or ingredient causes allergic reactions, irritation or sensitization on the skin [48]. The pilot patch study confirmed that the product is well tolerated by the skin, with no visible erythema or edema observed at 48 hours

for any participant. No signs of irritation or allergic reactions were observed during the study. The pilot study results support that F10 has good short-term cutaneous tolerance.

CONCLUSIONS

This study successfully achieved its objective of developing a hemp seed oil-based makeup remover balm with satisfactory physicochemical characteristics, microbiological quality, and physical stability. Among the ten formulations evaluated, F10 demonstrated the best overall performance, exhibiting good texture homogeneity, consistency, rinsability, and stability throughout the study. The formulation also showed acceptable microbiological quality and good skin compatibility in the pilot patch test, supporting its potential as a cosmetic cleansing product. According to the findings:

- careful optimization of the wax-oil ratio, emulsifier selection, and processing conditions is essential for developing stable, effective anhydrous cleansing balms containing hemp seed oil;
- achieving a uniform oil phase dispersion could be considered a key formulation objective, as it contributes to both product stability and desirable application characteristics;
- future studies could incorporate comprehensive chemical stability and performance of the cleansing balms, such as assessing the degradation of the fragile polyunsaturated fatty acids in hemp oil, and dermatological evaluations of the formulations in terms of their potential for *in vivo* cleansing efficacy, prone to skin pore clogging, acne development, and comedogenicity;
- broader safety studies with larger sample sizes and comprehensive user-based evaluations are recommended to support product optimization and to gather feedback on real-world application preferences.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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AUTHORS' CONTRIBUTIONS

Conceptualization: IK, GY; Methodology: IK, GY; Supervision: GY; Writing – original draft: IK, GY; Writing – review & editing IK, GY.

ETHICS STATEMENT

The dermatological pilot patch test was conducted at SKINLAB P.S.A. in Kraków, Poland. The patch test was conducted in accordance with applicable regulatory and ethical requirements stated in the methods section under the constant care of a dermatologist. All assessments, including volunteer qualification, sample application, and result interpretation, were conducted on-site at SKINLAB P.S.A. in Kraków.

USE OF AI TOOLS

The manuscript was reviewed for English language accuracy and grammatical consistency using the Grammarly AI tool provided by Yeditepe University and ChatGPT (OpenAI). The authors reviewed and edited the output and take full responsibility for the article's content.

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