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Goji berries (*Lycium barbarum*) as new potential cosmetic ingredient – A first screening

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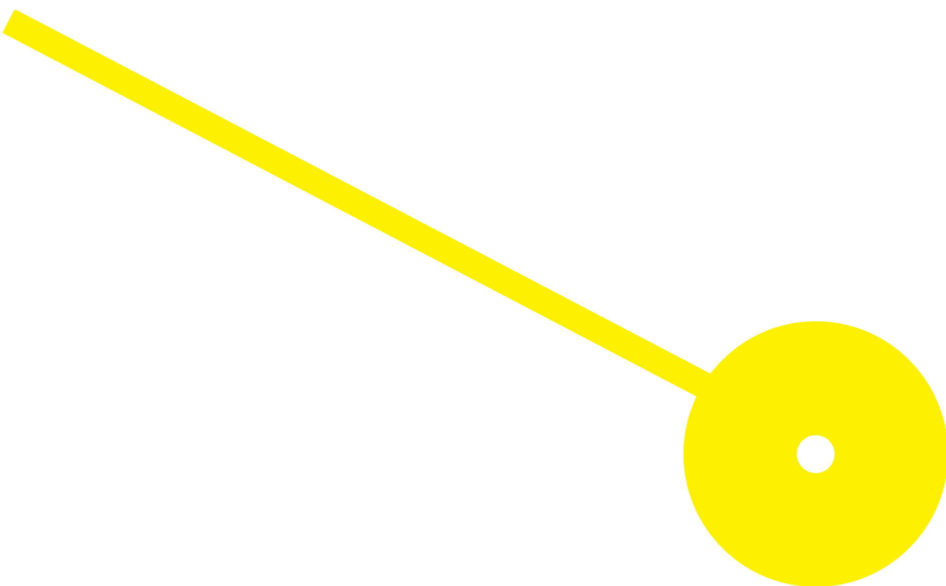
09/**2024**



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Goji berries (*Lycium barbarum*) as new potential cosmetic ingredient – A first screening

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Dissertação apresentada para cumprimento dos requisitos necessários à obtenção do grau de Mestre em **Bioquímica em Saúde – Ramo em Bioquímica Clínica e Metabólica** pela Escola Superior de Saúde do Instituto Politécnico do Porto.

Acknowledgements

First, to my supervisors, thank you for accepting this project and for receiving me with open arms. To Professor Francisca Rodrigues and Professor Helena Amaral. For all the patience, all the knowledge, my sincerest thanks to you.

A major thanks you to everyone I had the pleasure to work with, especially Ana Margarida Silva and Filipa Teixeira. Your help was vital for this project.

To my friends, without you I couldn't have made it. Thank you for all the hours spent listening to me, even when it was repetitive. Thank you for all the moments we spent together, I will keep you in my heart forever. Pedro, Kika, Ines Alex, you are very special to me and the best people I could have by my side. For all the gossip sessions, for all the crying sessions and for all the study sessions. I know I can always count on you.

To Teresa, who even when she didn't understand anything offered me help, who never left me alone, who always encouraged me and reminded me that I could do it.

To Ricardo, who supported me every time I fell, who always gave me the best hugs, and was my safe haven. I love you!

And lastly, the most special people in the entire world, my family. My parents who made so many sacrifices so I could be here, a thousand thanks. Without your support it would have been impossible. To my brother for being my partner from day 1 and who I can trust 100%. Thanks, maninho, for always being with me.

Resumo

Atualmente existe uma enorme preocupação com a aparência, tendo aumentando o número de produtos disponíveis no mercado para esta finalidade. Embora os produtos cosméticos não sejam comumente associados a problemas de saúde nefastos, os efeitos indesejáveis podem ocorrer devido à presença de ingredientes alergénicos, tendo vindo a ser cada vez maior o interesse por ingredientes naturais e à base de plantas. Entre os compostos naturais, as bagas de goji surgem como uma fonte interessante de compostos bioativos devido à sua riqueza em fenólicos, carotenoides, ácidos orgânicos, hidratos de carbono e vitaminas, muito associados à sua atividade antioxidante, antimicrobiana e anti-inflamatória. Deste modo, o principal objetivo deste estudo foi desenvolver uma formulação cosmética com extrato de bagas de goji obtido através de extração assistida por ultrassons.

O estudo de viabilidade celular efetuado em queratinócitos revelou que o extrato é seguro até à concentração de 1000 ug/mL. O ensaio de permeação *ex-vivo* realizado em pele humana durante 24 horas permitiu verificar a permeação de diversos compostos tais como o ácido 3,5-dicafeoilquínico, a rutina e a isorhamnetina-3-*O*-neohesperidosídeo, os quais atingiram às 24 h percentagens de permeação de 82,45%, 58,36% e 37,49%, respetivamente. De seguida, foram desenvolvidas formulações hidrofílicas incorporando o extrato de bagas de goji, sendo avaliada a sua estabilidade acelerada, pH, cor, textura e reologia. Os resultados permitiram verificar que as formulações têm propriedades físico-químicas adequadas para aplicação na pele.

Deste modo, o presente trabalho permitiu concluir que a emulsão múltipla gelificada com extrato de bagas de goji desenvolvida é um produto cosmético promissor para a prevenção do envelhecimento cutâneo. No entanto, para atestar este efeito, terão de ser efetuados ensaios biométricos em voluntários humanos, sendo igualmente interessante realizar estudos de estabilidade por um período alargado de tempo.

Palavras-chave: Bagas de goji, permeação, formulações cosméticas.

Abstract

Currently there is a great concern regarding skin appearance and new skin care products. Although cosmetic products are not commonly associated with serious health problems, potential undesirable effects may occur due to the presence of allergenic ingredients. Therefore, natural and plant-based ingredients are emerging as promising active ingredients with pro-healthy skin effects, attracting the consumer interest. Among natural sources, goji berries appear as an interesting source of bioactive compounds due to its richness in phenolics, carotenoids, organic acids, carbohydrates, and vitamins. Its antioxidant, antimicrobial and anti-inflammatory activities make goji berries promising for cosmetic applications. The aim of this study was to develop a cosmetic formulation with an ecological extract obtained from goji berries through ultrasound-assisted extraction.

The cell viability studies were performed on skin cell lines, namely keratinocytes, and revealed that doses up to 1000ug/mL were safe. An *ex-vivo* permeation assay was performed on human skin during 24 hours, being possible to observe that different compounds such as 3,5-dicaffeoylquinic acid, rutin, and isorhamnetin-3-*O*-neohesperidoside, achieved high skin permeation levels after 24 hours, respectively 82.45%, 58.36%, and 37.49%.

Afterwards, hydrophilic formulations incorporating goji berries extract were prepared and accelerated stability studies, pH, color, texture and rheology were evaluated. The formulations presented physicochemical properties suitable for skin application and promising stability characteristics.

The work allowed to conclude that, in general, the gelled multiple emulsion containing goji berries extract is an auspicious cosmetic product for skin aging prevention. As future perspective, it is necessary to perform biometric tests on human volunteers to confirm this property and it will be also interesting to carry out stability studies for a longer period.

Keywords: Goji berries; cosmetics; permeation; cosmetic formulations.

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List of Abreviations

DMEM – Dulbecco's Modified Eagle Medium

DMSO – dimethyl sulfoxide

HBSS – Hank's Balanced Salt Solution

MAE – microwave-assisted extraction

MTT – 3-[4,5dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide

PIF – Product Information File

SFE – supercritical fluid extraction

SWE – subcritical water extraction

UAE – ultrasound-assisted extraction

UV – ultraviolet

W/O/W – water-in-oil-in-water

1. Introduction

The knowledge about plants has accompanied the evolution of humans through time. From the earliest age, the most primitive civilizations used plants for food and health (1). The oldest record of plants employment in therapy refers to the Homo Neanderthal found in a cave on the border between Iraq and Iran, where the body was surrounded by eight species of plants (1). In recent civilizations, the contribution of Hellenic people stands out through Hippocrates, Galen, and Theofastus, responsible for the "History of Plants", with precise botanical descriptions and mention of toxic effects and healing properties (1).

Herbal products have been utilized for centuries across various cultures due to their medicinal and therapeutic properties (2). In recent years, there has been a significant resurgence in the popularity of herbal ingredients in skincare and cosmetic industry, driven not only by a growing consumer demand, but also for sustainable alternatives to synthetic chemicals (2,3). Skincare and cosmetics play a vital role in personal care routines, impacting not only physical appearance, but also psychological well-being (2,4,5). Consumers became increasingly aware about the cosmetic products ingredients, demanding for formulations that are not only effective but safe (5). The inclusion of herbal products in skincare and cosmetics offers numerous benefits, such as antioxidant, anti-inflammatory, and antimicrobial properties (6).

1.1. *Lycium barbarum*

Lycium barbarum, commonly known as goji berries (Figure 1), are small, red-orange fruits that resemble raisins when dried (7). They have a slightly tangy or sweet taste (8,9). Goji berries (figure 1) have been cultivated and consumed for over 2,000 years in different regions with temperate climates of Asia, particularly China, Tibet, and Mongolia (10). In traditional Chinese medicine, goji berries are considered a tonic herb with various health-promoting properties (7,11), being associated with the improvement of vision, boost immune function, and promoting longevity (12). Additionally, goji berries hold cultural significance in Asian countries, often associated with good health, prosperity, and longevity.

Figure 1. *L. barbarum L.*berries (13)

These berries are rich in antioxidants, particularly vitamin C, beta-carotene, zeaxanthin, and various flavonoids, which may neutralize harmful free radicals and protect cells from oxidative damage (13,14). Vitamins A, B2, B6, and E, as well as essential minerals like iron, zinc, and selenium have also been identified in their composition (11). Moreover, goji berries are low in calories and offer a moderate amount of a carbohydrates, fibbers, and proteins (15).

Several potential health benefits have been associated with goji berries intake, including immune support, eye health promotion, anti-inflammatory effects, and cardiovascular health improvement (7,16–18). The high antioxidant content of goji berries may bolster immune function and protect against infections, while compounds like zeaxanthin and beta-carotene support the eyes health and may help preventing age-related macular degeneration (14,19). Moreover, the anti-inflammatory properties may help to reduce inflammation and alleviate symptoms of certain chronic conditions (19). Additionally, the consumption of goji berries has been associated with the improvement of cardiovascular health, including lower blood pressure and cholesterol levels (18).

Commonly consumed as dried fruits, juice, or dietary supplements, goji berries have gained recognition as a "superfood" in health and wellness industries due to their nutrient density and potential health benefits (14,20).

1.2. Bioactive compounds

Bioactive compounds are substances found in plants, animals, and medicines that may contribute to the prevention and treatment of diseases, such as cancer and diabetes (21). Goji

berries are rich in carotenoids, vitamins (A, B1, B2, B3, B6, C), polysaccharides, zinc, and some amino acids (proline, serine), compounds with well-established skin effects (table 1) (9,14,15,22).

Table 1. Composition of goji berries and their biologic properties

Goji berries bioactive compounds	Biological Effects	References
Carotenoids	Protect the skin barrier and scavenge wrinkle-causing free radicals	Ma et al. (9) Vidovic et al. (14)
Vitamin A	Promote cell renewal and maintain skin radiant	Ma et al. (9)
Vitamin B1, B2, B3, B6	Help to maintain moisture, reduce fine lines, flaking and wrinkles	Teixeira et al. (15)
Vitamin C	Keep skin elastic	Vidovic et al. (14)
Polysaccharides	Antioxidant properties and protection against pollutants	Ma et al. (9)
Zinc	Regulate skin oil production	Vidovic et al. (14)
Amino acids	Keep skin soft	Ma et al. (9) Vidovic et al. (14)

Carotenoids have a powerful antioxidant property that helps to protect the skin barrier and fight wrinkle-causing free radicals (23). Berries are especially rich in zeaxanthin and lutein, which are essential for healthy vision, protecting the eyes from ultraviolet (UV) rays and the blue light emitted by phones and computers (23). Lycopene, a skin brightener, is also present in goji berries (24). Some carotenoids, such as cryptoxanthin and beta-carotene, are also present in goji berries, being converted into vitamin A, or retinol, within the body (25). When ingested, vitamin A helps to promote cell renewal, keeping skin radiant and allowing skin and mucous membranes to repel bacteria and viruses more easily (24). Vitamins B1, B2, B3 to B6 are essential for converting food into fuel to keep body energized and alert (26). Goji berries provide nearly 100% of the daily B2 intake, which helps maintaining the optimal immune and digestive function (27). These

vitamins also help the skin to retain moisture, reducing fine lines, flaking and wrinkles (27). Vitamin C is essential for keeping the immune system strong and promotes the collagen production, ensuring the skin elastic (28,29). Polysaccharides are molecules that help to maintain the optimal energy levels and reduce inflammation (14). Goji berries contain huge amounts of polysaccharides, which also have antioxidant properties, protecting skin from pollutants and allowing it to retain better hydration (9,28). Zinc helps to reduce inflammation and regulate the skin oil production , making it particularly useful for people prone to acne, psoriasis, and eczema (24). This metal has also capacity to promote the production of collagen and elastin, contributing to skin elastic and youthful (24). Goji berries are packed with amino acids, which help skin hydration and allow collagen, elastin, and other proteins to connect each other fully and effectively, maintaining skin soft (28). Moreover, amino acids can help to protect against UV rays and allow a faster wound healing (18).

All these characteristics make goji berries quite interesting from a cosmetic point of view. Therefore, the aim of this study was to study an extract obtained from goji berries through ultrasound-assisted extraction as new cosmetic ingredient incorporated in a topical formulation.

1.3. Green extraction techniques

Green extraction techniques represent a paradigm shift in the field of natural product extraction, promoting sustainability, efficiency, and environmental awareness (30). These approaches prioritize reducing environmental impact when compared to traditional methods, which often result in low yields, substantial use of toxic solvents, and degradation of heat-sensitive compounds (30,31). Green extraction offers benefits such as increased efficiency, reduced solvent consumption, and preservation of valuable bioactive compounds (31). Green extraction techniques include supercritical fluid extraction (SFE), subcritical water extraction (SWE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) (32,33). Each of these techniques brings unique advantages, offering more sustainable and environmentally friendly solutions for the extraction of bioactive natural products (30,31).

Supercritical fluid extraction (SFE) is notable for being an environmentally friendly technique that uses supercritical fluids – substances at temperatures and pressures above their critical points – to extract desired compounds from natural materials (32,34). Carbon dioxide is the most widely used supercritical fluid in SFE, due to its non-flammable and non-toxic properties, which ensure

a safe and environmentally friendly process(34). Compared to conventional extraction methods, SFE significantly reduces waste production and offers superior efficiency (32,34).

Subcritical water extraction (SWE) is an innovative method that uses heated water as a solvent to extract bioactive natural products from diverse matrices, such as food and agricultural by-products (34). The versatility of water as a solvent, capable of dissolving polar and non-polar compounds, makes SWE a highly efficient technique (34). In addition, it allows the selective extraction of phytocompounds with specific biological activities (34). Compared to steam distillation, SWE offers advantages in terms of time and energy consumption, preserving thermally sensitive compounds and ensuring a more sustainable extraction (34).

Ultrasonic-assisted extraction (UAE) involves the use of a piezoelectric transducer, which emits ultrasonic waves to agitate the solvent and improve extraction efficiency (32–35). There are two main types of UAE systems: probe and bath (32,34). Ultrasonic probe tends to be more efficient than bath, due to the higher ultrasonic intensity at the probe point, making it an effective tool for the extraction of bioactive compounds (34,35). UAE has been highlighted for its ability to extract compounds such as pectins, polyphenols and oils from fruits, vegetables and agricultural waste, consolidating itself as a promising technique for the extraction of natural compounds (32,33,35,36).

Microwave-assisted extraction (MAE) combines microwave energy with solvents, accelerating the extraction by heating the materials and solvents to specific frequencies (300 MHz to 300 GHz) (34). This direct heating increases the efficiency of the process, while preserving the biological activity of the extracted compounds (32). An example of successful use of MAE is the improvement of the extraction of green tea compounds, where it was possible to increase the antioxidant activity of phytocompounds and improve the colour quality and phenolic content of the extracts (32).

Although several sustainable extraction techniques are available, UAE is widely considered by the scientific community to be the most promising (37,38) and, for this reason, was the technique selected for this project. UAE offers an ideal combination of efficiency, solvent savings and preservation of bioactive compounds from goji berries (37,38) .

Once the most appropriate extraction method to preserve the bioactive compounds from goji berries has been selected, it is essential to understand the regulatory framework for cosmetics and the procedures required for the safe and effective introduction of new ingredients into the market.

1.4. Cosmetic regulation and validation of new active ingredients

The development and marketing of cosmetic products, including the incorporation of new active ingredients, are strictly regulated to ensure the safety and efficacy of the products for the consumer. In the European Union, the main legislative framework regulating cosmetics is Regulation (EC) No. 1223/2009 (39–41), which sets out guidelines for the safety of cosmetics products, applicable to all member countries of the union (39–41).

According to Regulation (EC) no1223/2009 of the European legislation on cosmetic products of 30 November 2009, a cosmetic product can be defined as "*any substance or mixture intended to be placed in contact with external parts of the human body (epidermis, hair system, nails, lips, and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours*" (42).

This regulation not only specifies what is a cosmetic, but also requires that all products placed on the European market are safe for human use (42). To this end, each cosmetic product must be accompanied by Product Information File (PIF) composed by: product formula, with identification of each ingredient; safety assessment, which contains a toxicological analysis of each active ingredient and an assessment of potential risks to human health; efficacy studies, which prove the benefits that the product claims to offer; description of the manufacturing method and compliance with good manufacturing practices standards (43).

The introduction of a new active ingredient, such as goji berry extract, in a cosmetic formulation requires a rigorous validation process (43). This process begins with the complete chemical identification of the ingredient, followed by a safety assessment to ensure that the new compounds does not pose a risk to the consumer's health (39). Particularly, toxicological assessment is a critical step in the validation of new ingredients and involves a thorough analysis of their toxicity (40). This includes:

1. Acute and chronic toxicity: assessed the adverse effects of short- and long-term exposures (40).
2. Genotoxicity and mutagenicity: tests the ingredient's ability to cause damage to genetic material (40).
3. Skin irritation and sensitization: checks whether the ingredient can cause irritation or allergic reactions on the skin (40).

The evaluation of the ingredient safety also depends on its skin absorption, i.e. the amount of ingredient that can penetrate the skin layers and reach the bloodstream (39). This aspect is essential, especially in the case of ingredients with potential systemic risks, since a cosmetic ingredient is generally expected to be safe only for topical application (39).

Since 2013, the European legislation completely banned the animal testing for cosmetic ingredients and finished products, as well as the sale of cosmetics that have been tested on animals outside Europe (40,44). Therefore, *in vitro* and *in silico* methods (based on computer simulations) are preferably used to assess the safety of new ingredients (39).

In addition to safety, efficacy validation is a crucial requirement for new cosmetic ingredients. A cosmetic product must be able to live up to the claims it makes, such as anti-aging, moisturizing, or antioxidant (39) . To this end, *in vitro* and *in vivo* studies are carried out, where the efficacy of the active ingredients is tested under laboratory conditions and on human volunteers (39,45).

The regulatory and validation process for new cosmetic ingredients is therefore comprehensive and involves not only verifying the safety of the active ingredient, but also proving the benefits it promises to offer (39). Compliance with European legislation and the use of non-toxic and sustainable methods are essential for the introduction of new ingredients, such as goji berry extracts, into the cosmetics market (39). These processes ensure that the final product is safe, effective and ethical, both for consumer and environment (41).

After undergoing a rigorous regulatory process, goji berry extracts can be introduced as safe and effective new ingredients. From here, the development of innovative cosmetic products can exploit the full potential of these compounds, aligning with the trends of natural and functional cosmetics.

1.5. Development of new cosmetic ingredients

The cosmetic industry is characterized by the continuous development of new products that seek to meet the growing demands of consumers in terms of efficacy and safety (39). With the increase in environmental awareness and the demand for healthier products, trends in organic and functional cosmetics stand out today, which seek to offer benefits beyond basic aesthetics, involving properties that improve the skin health (46,47).

Organic cosmetics are one of the fastest-growing categories in the market, driven by a growing demand for natural and sustainable products (46–48). These cosmetics are formulated with ingredients from organic farming and minimally processed, without the use of pesticides,

synthetic fertilizers or genetically modified organisms (46). In addition, they avoid the use of artificial preservatives, synthetic fragrances, and other potentially harmful chemical compounds (46). However, the development of organic cosmetics brings challenges (46). Preserving organic products without resorting to artificial preservatives, and maintaining the effectiveness of natural ingredients over time, are problems that manufacturers need to solve through innovative formulation methods (46).

Functional cosmetics are formulated with active ingredients that promote specific benefits to the skin beyond superficial appearance (49). These products are not limited to improving aesthetics, but also aim to treat dermatological problems such as acne, hyperpigmentation, signs of aging or sensitive skin (49). These cosmetics often contain bioactive ingredients such as peptides, antioxidants, hyaluronic acid or natural extracts such as goji berries. Scientific research is often used to prove the effectiveness of these ingredients, making them particularly attractive to consumers looking for tangible results (49).

The development of new cosmetic products involves a multifaceted process, from initial research to final market positioning (50). This process can be divided into several main phases:

1. Market research: is an essential step in identifying consumer needs and expectations (51). Cosmetic companies conduct market research to understand trends, consumer preferences and gaps in the market that new products can fill. The research helps determine the type of product to be developed, such as an anti-aging cream or a hydrating serum with natural ingredients (51). In addition, at this stage, it is important to analyse the competition and identify opportunities for innovation that can differentiate the product from the competition (51). Market research also guides companies on regulatory requirements in different regions (51).
2. Formulation: this phase involves developing a composition that meets the product's objectives, such as hydration, rejuvenation or antioxidant protection. In the case of organic and functional cosmetics, the ingredients must be carefully selected to ensure that they meet the efficacy and safety requirements (50). Organic cosmetics must contain natural ingredients and certified organic origin. Functional cosmetics require the incorporation of active ingredients that have been proven to deliver the promised benefits, such as antioxidants from goji berries (49). The formulation process must also ensure that the active ingredients are stable over time and do not interact negatively with each other (52).

3. Stability and Compatibility Tests: after formulation, stability tests are performed to ensure that the product maintains its properties throughout its shelf life (52). These tests simulate different storage conditions, such as temperature and light, to ensure that the product remains safe and effective (52). Cosmetic products also undergo compatibility tests to ensure that the product does not react adversely with the packaging and other components (52). Stability tests check for aspects such as changes in colour, texture and odour; phase separation (in emulsions, for example); degradation of active ingredients; changes in pH (52). Compatibility tests assess the interaction of the product with the packaging materials, ensuring that the product does not degrade or contaminate the container and vice versa (52).
4. Marketing and Positioning: marketing and positioning of the product are crucial to its success in the market (53). This stage involves defining communication strategies, choosing distribution channels and defining the target audience (53). Positioning focuses on highlighting the competitive advantages of the product, such as its efficacy, organic origin, natural ingredients or functional benefits, and aligning the product image with consumer trends (53). Particularly, in the case of organic and functional cosmetics, marketing often emphasizes:
    a. Sustainability: The use of organic ingredients and environmentally friendly manufacturing practices (51).
    b. Health and Wellbeing: Products that do not contain harmful substances and offer real benefits to the skin (43).
    c. Proven efficacy: For functional cosmetics, clinical studies and scientific evidence are often used as selling points (49).
    d. Creating a strong brand identity, combined with building a narrative that resonates with consumers' environmental and health concerns, is a key factor in product acceptance in the market (53,54).

The development of new cosmetic products, particularly in the area of organic and functional cosmetics, involves a complex process that ranges from market research to formulation and rigorous stability testing (52). The growing demand for products that combine sustainability with functional benefits creates opportunities for natural ingredients such as goji berries, whose antioxidant and anti-aging potential make them attractive for today's cosmetics market (51). To

ensure the success of a new product, it is crucial to align all stages of development with consumer expectations and global market demands.

2. Objectives

This project aims to study an eco-friendly extract obtained from goji berries through ultrasound-assisted extraction as new cosmetic ingredient incorporated into a cosmetic formulation. For this purpose, the specific objectives are:

- To assess the toxicity of the goji berries extract on keratinocytes;
- Evaluate ex-vivo permeation of goji berries.
- To develop a hydrophilic cream and a gelled multiple emulsion with and without the extract;
- To evaluate the stability of the hydrophilic cream and the gelled multiple emulsion, with and without the extract, regarding droplet size, pH, accelerated stability by centrifugation, color, rheology and texture.

3. Materials and Methods

3.1. Materials

Chemicals and reagents were acquired from Acofarma (Spain), Guinama (Spain), Lubrizol (Belgium), and Fagron (Spain). Cells reagents were supplied by Invitrogen Corporation (Spain). The human skin cells were provided from American Type Culture Collection (United States of America).

3.2. Goji berries Ultrasound-Assisted Extraction

Goji berries were weighted and diluted in water in a ratio 8.47:100 (*m/v*), according to the conditions reported by Teixeira *et al.* (55). Briefly, the UAE was carried out in an ultrasonic probe processor (Sonic Vibracell, model VCX50, Newtown, CT, USA) equipped with a probe tip No.630-0219, featuring a 13 mm diameter and a frequency of 20 KHz. The ultrasonic time was 56 minutes and 21 seconds with an ultrasonic intensity of 59.05 W/m^2.

Afterwards, samples were centrifuged (Megafuse 16R centrifuge, Thermo Fisher Scientific, Germany) at 5000 rpm at 20 °C for 30 minutes and the supernatants were filtered through a Whatman nº2 paper and frozen (Haier Biomedical, China) at -80 °C for subsequent lyophilization (Telstar, model Cryodos-80, Barcelona, Spain) (Figure 2).

Figure 2. Scheme of extraction process of *L. barbarum* berries

3.3. Cell viability assays

The cell viability assays were performed using skin cell lines, namely keratinocytes (HaCaT), to evaluate the viability potential of goji berries extract. The cell lines were cultivated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% fetal bovine serum, 1% non-essential amino acids, and 1% Penicillin-Streptomycin. Cells were incubated in a 5% CO_2 environment at 37 °C (ESCO GB Ltd, UK). The vital mitochondrial dye 3-(4,5-dimethylthiazol-2-yl)-2,5-

diphenyltetrazolium bromide (MTT) assay followed the methodology described by Pinto *et al.* (126). The medium was removed, and the cells washed using Hank's Balanced Salt Solution (HBSS). Afterwards trypsin was added, and the medium incubated for 5 to 15 minutes. After neutralizing with trypsin, the samples will be centrifuged at 1500 rpm for 20 minutes, at 20 °C. The supernatant was discarded, and the pellet diluted to count the cell and posterior seeding them in 96-well plates (2 x 10^4 cells/well) for 24 h. Afterwards, the medium was removed, and the cells were exposed for 24 h to different extract concentrations (1.0 – 0.03125 mg/mL). After 24 h of incubation, samples were removed, and the cells washed with HBSS. The number of viable cells was determined by adding the MTT reagent and incubating for 3 h, at 37 °C, allowing the formation of formazan crystals. After incubation, dimethyl sulfoxide (DMSO) was added, for 10 min, to solubilize the crystals. The absorbance (BioTek Instruments, Synergy HT, USA) was read at 590 nm, with a background subtraction at 630 nm. Triton X-100 1% (*w/v*) and DMEM were used as negative and positive controls, respectively.

3.4. *Ex-vivo* skin permeation

Human skin was obtained from a female patient, undergoing an abdominoplasty. The experimental protocol was approved by the Bioethics Committee of the São João Hospital and a written informed consent form was provided to the volunteer. The skin was frozen at -20 °C and when needed defrosted at room temperature immediately before use. The Franz diffusion cell system was used to test the penetration of compounds present in goji berries. A Franz cell assembly (9 mm unjacketed Franz Diffusion Cell) comprising a 5mL receptor volume and a diffusion area of 0.8 cm^2 (PermeGear, Inc. Pennsylvania USA) was used. The skin cells were placed between the donor and the receptor phase with the *stratum corneum* side facing upward into de donor compartment (Figure 3). The donor medium consisted of 300 µL (500 µg/mL). The receptor (5 mL) was filled with PBS buffer, The stirring rate and temperature of receptor solution were, respectively, 600 rpm and 37 °C. At appropriate intervals (15 to 1440 mins) 300 µL aliquots of the receptor medium were withdrawn with a syringe and immediately replaced with equal volumes of fresh receptor phase. The cumulative quantity of compounds will be determined by LC-MS.

LC-MS analysis, previously reported by *Silva et al.* (56), was performed using an Agilent 1260 chromatographic system equipped with diode array detector and Varian MS 500 Ion trap. As stationary phase an Agilent C-18 Eclipse column 3.00 × 150 mm (3.5 µm) was used. The eluents

will be water 1% formic acid (A) and acetonitrile (B). Gradient elution started from 10% B going in 30 min to 100% A, then isocratic for 5 min and equilibration time to 90% A until 40 min. The flow rate was 400 µL/min. After column, the liquid will be equally splitted with a "T" connector to diode array detector and MS. Spectra will be acquired in the range 100–2000 Da in negative ion mode. To generate fragmentation of most intense ion species the turbo data detection scanning (tdds) function of the instrument will be used. The MS parameters were the following: drying gas temperature 310 °C, drying gas pressure 25 psi, nebulized 30 psi, capillary 100 V, needle 4500 V, shield 500 V, RF loading 80%.

Figure 3. Representation of the skin permeation assay.

3.5. Development and characterization of a cosmetic formulation

Different hydrophilic cream formulations were prepared (Table 2). To prepare the creams, the ingredients of the aqueous phase were mixed and heated using a water bath (Nahita, Auxilab, Navarra, Spain) at 70-80 °C. The oil phase was heated in the same conditions. When both phases were at the same temperature, the aqueous phase was added slowly to the oil phase with continuous stirring. The mixture was stirred until it reaches the room temperature (Figure 4).

Table 2. Composition of the hydrophilic creams (%, w/w).

	CR1	CR2	CR3	CR4
Ingredients	%(w/w)			
	Oil phase			
Lanette® N Wax	20.00	15.00	20.00	15.00
	Aqueous phase			
Glycerin	5.00	5.00	5.00	5.00

Butylated hydroxytoluene (BHT)	0.05	0.05	0.05	0.05
Phenonip®	0.50	0.50	0.50	0.50
Goji berries extract	-	-	3.00	3.00
Purified water	q.s.100.0			

q.s.: quantity sufficient

Figure 4. Before (left) and after (right) all the ingredients addition.

During the cream's preparation, some oxidation occurred when the extract was added. To improve the formulations stability a water-in-oil-in-water (W/O/W) multiple emulsion was prepared (Table 3). Phases I and II were heated using a water bath at 70-80 °C. When both phases were at the same temperature, phase I was added to phase II, resulting phase III. Phase IV was heated on a stirring plate and was added to phase III with continuous stirring (1000 rpm, 5 min). To increase the multiple emulsion consistency, 0.5% of Carbopol® 940 was added to the external aqueous phase. Triethanolamine was employed to adjust the pH.

Table 3. Composition of the W/O/W cream (%, *w/w*).

	Ingredients	**EMG5**	**EMG6**	**EMG7**	**EMG8**
Phase I	Extract	-	3.00	-	3.00
	Purified water	q.s.100.00			
Phase II	Span® 80	2.50	2.50	2.50	2.50

Phase III	Isopropyl myristate	25.00	25.00	25.00	25.00
	W/O emulsion	40.00	40.00	40.00	40.00
Phase IV	Tween® 80	2.50	2.50	2.50	2.50
	Phenonip®	0.50	0.50	0.50	0.50
	Purified water	q.s.100.00			
	Carbopol® 940	0.50	0.50	0.75	0.75

3.5.1 Characterization of the cosmetic formulations

3.5.1.1. Accelarated stability

In this test, 6 mL of the hydrophilic cream was placed in a Falcon tube and submitted to two cycles of centrifugation (Centrifuge 5804, Eppendorf, Germany) (Figure 5) for 30 min at 3500 rpm.

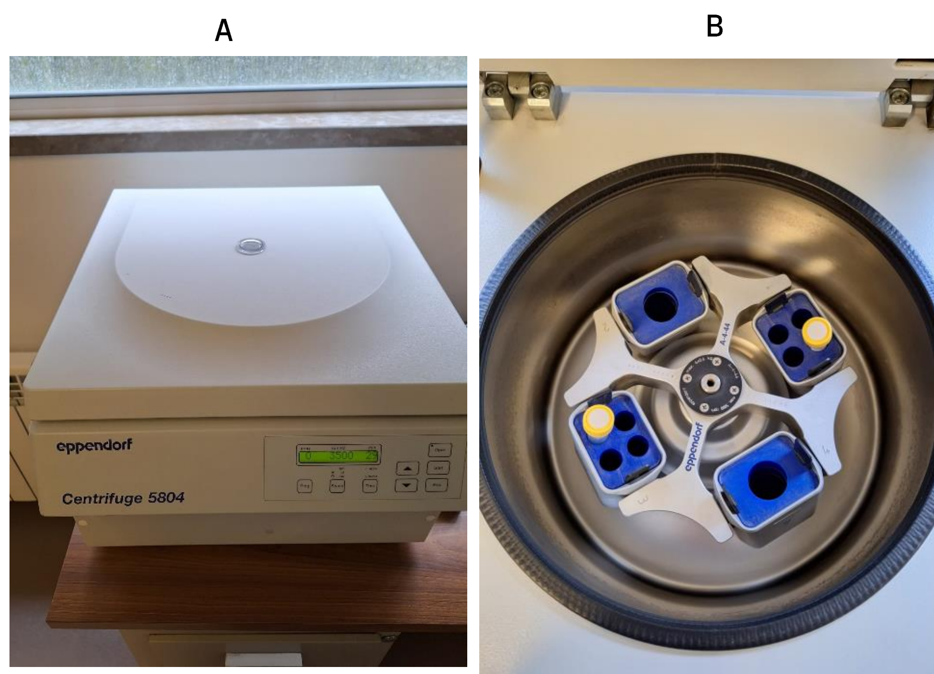


Figure 5. A. Centrifuge 5804, Eppendorf (Germany) B. Falcon tubes with the formulations.

3.5.1.2. pH

The pH is an important property of preparations for cutaneous application, since this parameter must be compatible with the pH body region where they will be applied. Moreover, the pH

determination over the storage time is one of the parameters that allows the assessment of the products stability.

In this study, the pH measurement was performed at 20 °C, in triplicate, after the preparation of the formulations, using a potentiometer (Basic 20, Crison Instruments, Spain) (Figure 6). The results shown correspond to the mean values of the 3 measurements ± the standard deviation.

Figure 6. Potentiometer Basic 20, Crison Instruments (Spain).

3.5.1.3. Color

To analyse the creams color, a colorimeter (Chroma meter CR-400, Konica Minolta, Japan) (Figure 7) was employed. The data was processed by the Spectra Magic TM NC software. The colorimeter was previously calibrated on a white surface (standard). The measurements were made in triplicate over 30 days after preparation of formulations.

Figure 7. Colorimeter Chromameter CR-400, Konica Minolta (Japan).

3.5.1.4. Texture

A texture analyser (TA-XT2i, Stable Micro Systems, United Kingdom) (Figure 8) was used to evaluate texture parameters and the data were processed by the Exponent software. The adhesiveness and firmness were evaluated using a 25 mm Perspex cylindrical probe. The test was performed in compression mode, with a 5 kg loading cell, a trigger force of 0.049 N, a penetration depth of 2 and 5 mm and a test speed of 3 mm/s. All measurements were made in triplicate over 30 days and the results were expressed as the mean ± standard deviation.

Figure 8. Texture analyser TA-XT2i, Stable Micro Systems (United Kingdom).

3.5.1..5 Rheology

Viscosity was evaluated by a viscometer (Thermo Haake Viscotester VT-550, Germany) (Figure 9) using a shear rate between 0.1 and $100s^{1}$, comprising 10 upward steps and 10 other descending steps.

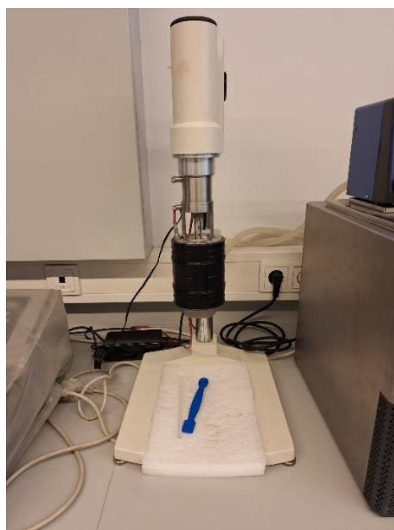


Figure 9. Viscometer Thermo Haake Viscotester VT-550 (Germany).

3.5.1.6. Measurement of droplets size

The droplet sizes of the oil phase of the W/O/W emulsions were evaluated using the Mastersizer 3000, Malvern, UK) (Figure 10) which uses the laser diffraction technique to measure droplets with dimensions between 10 nm and 3.5 mm.

The results of the droplet size assessment are generally presented in the form of a statistical distribution. These sizes are presented in percentiles, obtaining the distribution volume of 10%, 50%, and 90% (d10, D50 and D90 respectively), which refers to the droplets with diameters equal to or smaller than the values obtained. For each sample, the device takes several readings of the size and displays the average, the coefficient of variation and the *Span*.

The equipment parameters that were used in this work are shown in Table 4.

Table 4. Parameters used for measurement of droplets size.

Droplet type	Spherical
Material name	Lipid
Lipid refractive index	1.4
Density	1

Absorption index	0.01
Dispersant	Water
Dispersant refractive index	1.33

Figure 10. Laser diffractometer Mastersizer 3000, Malvern (United Kingdom).

3.6. Statistical Analysis

All measurements were performed in triplicate ($n = 3$) and the results were expressed as mean ± standard deviation (SD). A value of $p < 0.05$ was considered significant after using a one-way analysis of variance (ANOVA) followed by Tukey's HSD test, through Excel.

4. Results and Discussion

4.1. Cell's viability assays

In vitro assays are an accurate, fast and reproducible method to evaluate the effect of bioactive compounds in living cells. To determine if bioactive compounds can positively affect the cell proliferation or exert a cytotoxic effect, cell viability assays are of huge interest. In this work, the safety of the optimal goji berries extract was assessed by an MTT assay on keratynocites (HaCaT). The extract was tested in a concentrations ranging between 31.25 and 1000 µg/mL. Figure 11 summarized the results obtained.

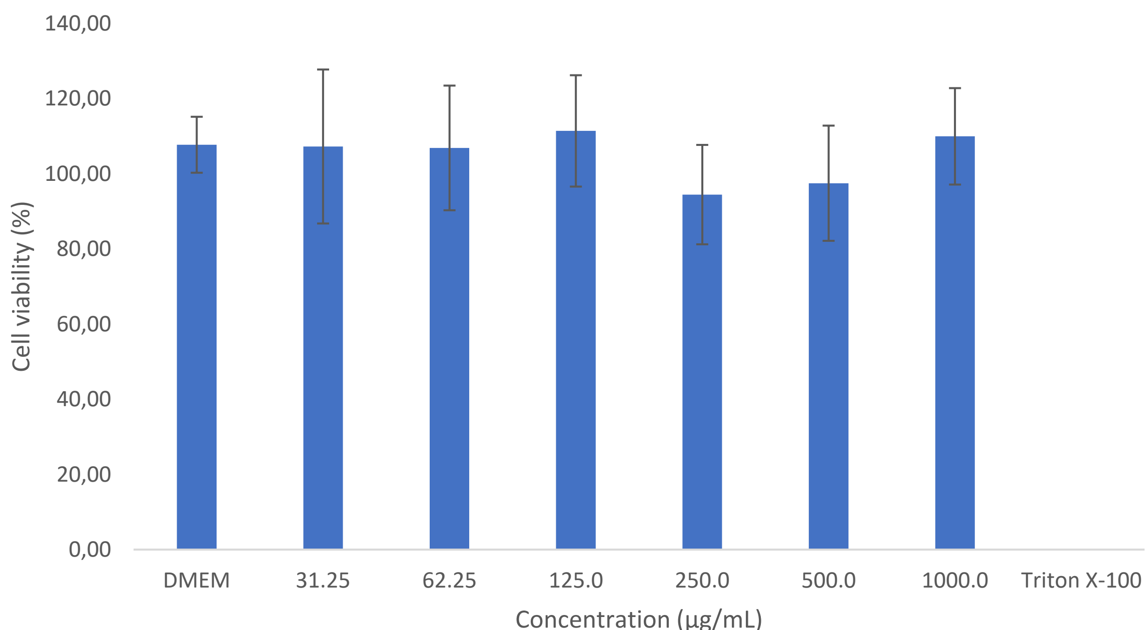


Figure 11. Effects of the exposure of the optimal *L. barbarum* berries extract on HaCaT viability at different concentrations (31.25-1000µg/mL). Results are expressed as mean ± standard deviation (*n*=3)

According to the obtained results, the optimal extract did not affect the viability of keratinocytes in concentrations lower than 1000 µg/mL, presenting results between 97% and 104%. Moreover, no significant differences were observed between concentrations. Therefore, this study emphasizes the non-cytotoxic effect of the optimal goji berries extract in concentrations below 1000 µg/mL in skin cell lines.

In the study conducted by Silva *et al.* (38)., aiming to evaluate the optimal conditions of antioxidants polyphenols from kiwiberry leaves using a response surface methodology, a cell viability assay was performed. the results show the optimal extract did not affect the HaCaT

viability, presenting results around 100% for all tested concentrations (ranging from 0.1 µg/mL to 1000 µg/mL). These results are accordingly to the ones mentioned above.

Cadiz-Gurrea *et al.* (57), proposed the possibility of using bioactive compounds from olive fruit and leaves as effective antioxidants with interesting skin health benefits. For this, a cell viability assay was performed. In this study, the HaCaT cell line viability did not decrease after exposure to different concentrations of oleuropein-enriched extracts, showing a viability increase of around 100% until a concentration of 100µg/mL. At 1000µg/mL there's a decrease in these values to approximately 60%. Thus, goji berry extracts appear to be better suited for incorporation into a cosmetic formulation.

4.2. *Ex-vivo* skin permeation

To identify which goji berries compounds were able to penetrate the skin, *ex vivo* experiments were conducted using human skin model. The different compounds identified are summarized in Table 5.

Table 5. Compounds identified in goji berries extract.

Formula	Identification	
$C_{16}H_{18}O_8$	Feruloylquinic acid	Phenolic acids
$C_{25}H_{24}O_{12}$	3,5 dicaffeoylquinic acid	
$C_{25}H_{24}O_{12}$	1,5 dicaffeoylquinic acid	
$C_{25}H_{24}O_{12}$	4,5 dicaffeoylquinic acid	
$C_{46}H_{71}N_4O_{21}$	3-glu-kukoamine-isomer	Others
$C_{40}H_{61}N_4O_{16}$	2-glu-kukoamine-isomer	
$C_{40}H_{61}N_4O_{16}$	2-glu-kukoamine-isomer	
$C_{43}H_{62}N_3O_{21}$	Glu-Lycibarbasperimidin-F-isomer	
$C_{43}H_{62}N_3O_{21}$	Glu-Lycibarbasperimidin-F-isomer	
$C_{31}H_{44}N_3O_{11}$	Lycibarbaspermidin B isomer	
$C_{31}H_{44}N_3O_{11}$	Lycibarbaspermidin B isomer	
$C_{31}H_{44}N_3O_{11}$	Lycibarbaspermidin B isomer	
$C_{25}H_{31}N_3O_6$	Dicaffeoyl spermidine	
$C_{25}H_{31}N_3O_6$	Dicaffeoul spermidine	
$C_{33}H_{40}O_{21}$	Rutin-hexoside	Flavonoids
$C_{27}H_{30}O_{16}$	Rutin	
$C_{27}H_{30}O_{16}$	Rutin isomer	
$C_{27}H_{30}O_{15}$	Kaempferol-3-*O*-rutinoside	
$C_{28}H_{32}O_{16}$	Isorhamnetin-3-*O*-neohesperidoside	
$C_{30}H_{48}O_4$	Corosolic acid	Others

Table 6 summarizes the different compounds permeation at each time point. As can be observed, a significant percentage of compounds permeated the skin. Several factors can influence the skin permeation of cosmetic ingredients, including, molecular size, compound structure, hydrophobicity, and permeation time (55). Compounds such as 3,5-dicaffeoylquinic acid, isorhamnetin-3-*O*-neohesperidoside, and rutin demonstrated high levels of skin permeation at the final endpoint of 24 hours, achieving permeation values of 82.45%, 37.49%, and 58.36%, respectively. These compounds are relatively small and have structural characteristics that allow them to cross the skin lipid barrier (55).

However, glycosylation may also play a key role in permeation, since the addition of sugar molecules increases the molecular size, which may hinder diffusion by common mechanisms (55). For example, compounds such as kaempferol-3-*O*-rutinoside, despite being glycosylated, achieved a final permeation of 36.95%. The increased hydrophobicity derived from glycosylation may have facilitated the passage through the skin. On the other hand, more hydrophobic compounds, such as corosolic acid, showed a substantially high permeation at the end point (76.82%), reflecting the ability of this compound to integrate into the lipid layers of the skin and cross it effectively. Another relevant example is glu-lycibarbarspermidine-F-isomer that achieved a permeation of 51.9% after 24 hours. This compound, being a spermidine, can exploit specialized transport mechanisms to cross the skin barrier, similar to what occurs with the intestinal absorption of polyamines (55). This capacity explains the relatively high permeation of other compounds with similar structures.

In contrast, dicaffeoyl spermidine showed a lower permeation, namely 45.22% after 24 hours, which may be related to its larger molecular size and its structure less conducive to passive diffusion through skin. Moreover, compounds such as 2-glu-kukoamine isomers reached permeation values of 79.19% and 87.21%, reinforcing the idea that the hydrophobicity associated with glycosylation may favor the skin permeation, even in compounds with higher molecular weight.

Furthermore, the time course permeation data show that many of the compounds reach a saturation phase after 6-8 hours of exposure. This suggests that cosmetic product formulations may benefit from adjusting the application time to ensure the efficacy of compounds that require longer permeation times.

Silva *et al.* (57), in a study with kiwiberry leaves, performed ex vivo skin permeation using Franz diffusion cell with human skin. In this study, the feruloylquinic acid was the principal phenolic

compound identified and quantified in the different time-points. However, the values were a little lower than the ones obtain in the work being described. Rutin also showed lower values of permeation.

Taofiq et al. (58), performed a permeation assay using the Franz diffusion cell with pig ear skin as the permeation membrane using a mushrrom ethanolic extracts. In this study, protocatechuic and syringic acids were the unique compounds that permeated. Protocatechuic has similar characteristics to feruloylquinic acid, and the permeation observer after 60 minutes is very similar. These authors report that the extract used can be utilised as an ingredient for cosmetical application due to its characteristics and because it penetrates the skin, offering advantages as an antioxidative support to the skin. Since feruloylquinic acid has similar characteristics, it is expected that it will have the same advantages.

Tabel 6. List of phytochemical compounds identified and quantified in the ex-vivo permeation through LC-MS analysis

Identification	t15	t30	t45	t60	t90	t120	t150	t180
Feruloylquinic acid	9.51±0.19	20.00±0.00	17.50±1.41	43.03±1.64	42.93±1.89	45.81±0.97	44.23±2.53	45.17±0.66
3,5 dicaffeoylquinic acid	7.23±0.13	8.04±0.00	12.30±1.13	51.29±1.05	54.98±0.31	47.70±3.66	54.95±2.04	66.08±0.82
1,5 dicaffeoylquinic acid	47.21±1.42	55.36±0.00	62.96±3.17	61.57±3.53	70.51±0.97	69.69±3.89	68.67±3.36	87.86±2.02
4,5 dicaffeoylquinic acid	5.78±0.21	0.44±0.00	0.49±0.10	0.94±0.08	12.75±1.56	2.13±0.39	3.33±0.15	3.36±0.25
3-glu-kukoamine-isomer	7.24±0.18	81.40±0.00	92.27±1.51	91.24±2.07	91.94±2.46	89.86±0.94	91.06±1.22	91.19±0.44
2-glu-kukoamine-isomer	1.10±0.21	12.84±0.00	43.50±1.99	47.86±0.49	44.88±3.30	52.69±2.67	57.22±0.12	58.00±1.51
2-glu-kukoamine-isomer	1.79±0.76	11.34±0.00	27.40±2.07	42.44±2.01	44.01±2.59	45.97±2.08	62.95±0.64	63.93±0.64
Glu-Lycibarbasperimidin-F-isomer	1.06±0.36	0.78±0.00	11.17±0.47	13.82±0.28	16.14±1.01	20.88±1.97	26.32±1.91	25.89±1.17
Glu-Lycibarbasperimidin-F-isomer	0.40±0.35	0.73±0.00	5.02±0.11	11.52±0.64	12.19±1.00	15.44±1.50	14.78±1.67	15.47±1.17
Lycibarbaspermidin B isomer	0.00±0.00	3.25±0.00	2.77±0.66	9.35±0.28	7.95±2.14	10.79±0.65	10.34±0.87	10.26±0.99
Lycibarbaspermidin B isomer	0.95±1.65	6.54±0.00	10.22±1.00	23.14±2.76	25.80±0.29	27.35±0.64	26.86±0.62	55.01±1.69
Lycibarbaspermidin B isomer	0.42±0.72	0.56±0.00	1.23±0.18	8.55±0.87	9.32±0.28	10.29±1.69	10.45±0.87	12.53±1.81
dicaffeoyl spermidine	0.62±0.87	0.26±0.00	9.10±0.82	15.22±1.38	15.43±1.16	31.57±1.50	46.49±4.20	93.91±1.01
dicaffeoul spermidine	4.01±0.28	4.47±0.00	3.63±0.06	5.51±0.50	5.50±0.54	14.40±0.92	20.91±0.72	43.19±1.99
rutin-hexoside	0.13±0.18	0.56±0.00	1.06±0.19	5.45±0.12	5.75±0.14	11.67±1.39	43.31±2.02	65.90±0.43
Rutin	5.67±0.76	7.83±0.00	26.48±1.67	48.05±1.20	49.54±1.90	50.75±1.76	52.32±2.66	57.98±1.29
rutin isómer	0.03±0.04	1.62±0.00	11.40±1.01	35.23±0.89	36.85±1.68	38.77±0.38	41.95±0.64	53.14±1.95
Kaempferol-3-O-rutinoside	2.17±0.31	2.52±0.00	16.26±1.07	18.31±0.85	18.30±1.73	18.10±1.15	19.65±0.64	29.82±0.53
Isorhamnetin-3-O-neohesperidoside	9.82±0.39	8.44±0.00	21.38±1.29	20.67±0.97	22.72±2.15	22.51±1.43	17.41±1.28	25.99±0.44
Corosolic acid	37.70±4.95	43.12±0.00	67.98±1.51<	70.38±2.01	72.02±0.68	70.35±1.91	68.98±4.92	72.29±1.12

Identification	t210	t240	t300	t360	t420	t480	t1440
Feruloylquinic acid	45.66±0.70	49.94±1.35	49.44±1.07	52.69±0.72	52.36±0.77	53.33±0.72	53.95±0.23
3,5 dicaffeoylquinic acid	61.38±1.59	65.34±0.20	66.45±0.35	71.88±0.88	70.88±0.96	82.80±0.57	82.45±0.84
1,5 dicaffeoylquinic acid	88.80±0.57	80.79±0.65	69.15±4.74	80.30±0.71	81.69±2.14	80.85±0.65	81.91±0.36
4,5 dicaffeoylquinic acid	3.80±0.37	5.35±1.02	5.16±1.43	5.44±1.04	5.56±0.62	6.65±0.21	6.31±0.05
3-glu-kukoamine-isomer	89.86±0.55	90.11±0.09	89.90±0.57	94.25±0.66	93.64±0.99	93.39±0.58	87.21±0.47
2-glu-kukoamine-isomer	58.99±0.35	56.44±6.13	78.90±0.85	75.45±1.34	74.83±2.32	78.93±0.04	79.19±0.01
2-glu-kukoamine-isomer	64.18±1.44	61.07±0.66	68.12±1.31	69.84±0.48	64.74±0.77	81.53±0.18	84.21±0.01
Glu-Lycibarbasperimidin-F-isomer	26.34±1.04	31.99±2.16	35.74±1.08	51.21±3.66	50.90±4.10	51.51±0.26	51.90±0.57
Glu-Lycibarbasperimidin-F-isomer	17.43±0.97	21.99±2.28	28.83±0.10	34.85±1.46	35.35±1.46	39.06±0.48	39.21±0.86
Lycibarbaspermidin B isomer	10.64±1.21	12.27±1.38	12.87±0.35	14.27±0.90	14.46±0.78	16.43±0.33	16.28±0.53
Lycibarbaspermidin B isomer	66.57±**1.19**	71.00±1.06	78.54±1.26	79.05±3.18	78.20±2.29	74.25±0.36	72.92±0.28
Lycibarbaspermidin B isomer	14.56±1.07	12.84±1.40	13.21±1.73	13.49±2.00	13.42±2.05	13.53±2.00	13.15±0.40
dicaffeoyl spermidine	93.70±1.06	91.87±2.37	97.64±1.22	94.59±2.14	92.51±2.08	92.53±1.38	91.41±0.79
dicaffeoul spermidine	45.39±4.00	41.78±1.67	44.19±1.40	43.74±3.65	44.25±1.09	44.49±0.02	45.22±1.39
rutin-hexoside	65.48±0.25	64.03±2.46	65.81±0.84	64.23±**1.1**5	64.42±2.56	78.38±0.17	75.59±**1.14**
Rutin	62.13±1.30	57.56±1.50	57.82±1.45	62.00±0.71	51.85±0.92	64.79±2.24	58.36±**1.1**9
rutin isómer	50.80±0.57	53.98±0.31	55.02±2.45	55.20±0.98	56.70±1.81	59.50±0.85	61.38±1.35
Kaempferol-3-O-rutinoside	31.57±1.44	26.33±**1.1**7	29.24±0.79	27.08±0.55	36.97±2.50	36.71±0.27	36.95±0.07
Isorhamnetin-3-O-neohesperidoside	32.35±0.21	35.95±3.60	31.73±1.50	33.29±1.86	32.91±0.83	37.12±0.87	37.49±0.89
Corosolic acid	72.39±1.05	73.79±1.01	72.51±0.15	72.70±1.62	72.13±0.90	78.87±0.52	76.82±2.07

4.3. Accelerated stability

The stability of a product is related with its capacity to resist to physical and chemical changes during a determined period (52). Cosmetics, such as day-creams, have daily exposure to the environment (which may include variations in humidity, light, and temperature) (39). Therefore, the stability of these formulations needs to be well established (52). The accelerated stability test makes it possible, through centrifugation, to quickly mimic the formulation's behavior when subjected to variations that may occur during its period of usage (39). Several changes may occur, including coalescence, formation of sediments, and phase separation, among others.
After preparing the formulas presented in Tables 2 (hydrophilic creams) and 3 (W/O/W creams), the formulations were submitted to a centrifugation cycle. Figure 12 shows the results of the accelerated stability study.

Figure 12. Formulations after two cycles of centrifugation.

As can be observed, the formulations maintained their integrity after two cycles of centrifugation, with all formulations presenting the same results. These results are in line with another study that developed hand creams with coffee silverskin extract, a coffee byproduct (59). According to the authors, the formulations, after being submitted to 2 cycles of centrifugation at 3000 rpm for 30 minutes (the same conditions used this work), no phase separation was observed in any of the storage conditions (25 °C and 40 °C). Rodrigues *et al.* (60) incorporated extracts of coffee silverskin and *Medicago sativa* into a body cream and evaluated its stability. Once again, no phase separation was observed at the end of the centrifugation cycles. Moreover, these results agree with another study that developed nanosystems to improve the skin penetration of caffeine for

the treatment of cellulite (61). After centrifugation at 3000 rpm for 30 minutes, the microemulsions maintained the homogenous appearance that presented initially.

4.4. pH

The skin naturally presents an acidic pH ranging from 4.1 to 5.8, with some exceptions. This value is also dependent on external and physiological factors, such as age. Due to the extent use of topical formulations, the pH evaluation is important to assure that formulations do not affect the skin barrier. Figure 13 summarized the results of the pH values of the hydrophilic creams over 30 days of storage at 25 °C and 40 °C.

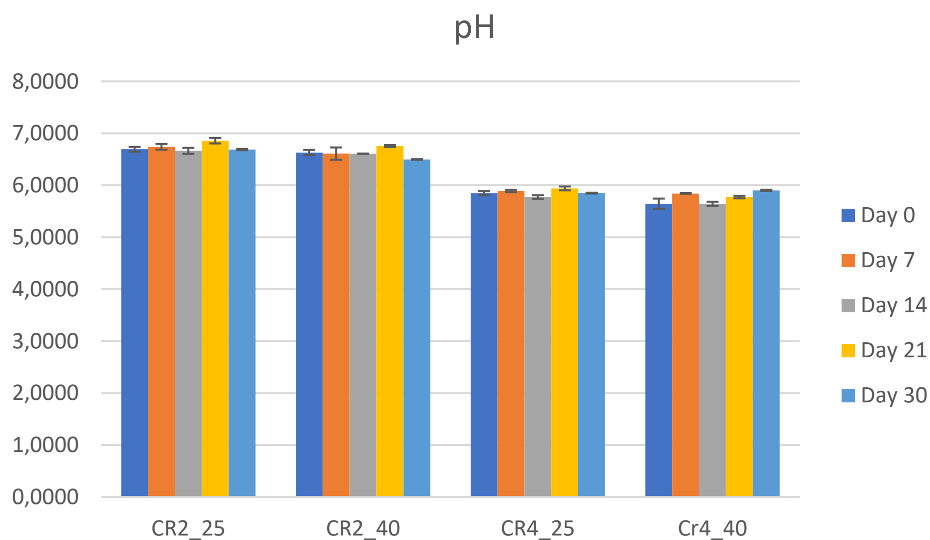


Figure 13. pH of formulations 2 (without extract) and 4 (with extract) over 30 days of storage at 25 °C and 40 °C.

The results show that the pH of both formulations is maintained over the 30 days. Moreover, the formulation with the extract (Formulation 4) presented a lower pH, suggesting that the extract is responsible for this decrease.

The pH values of W/O/W creams are summarized in Figures 14 and 15.

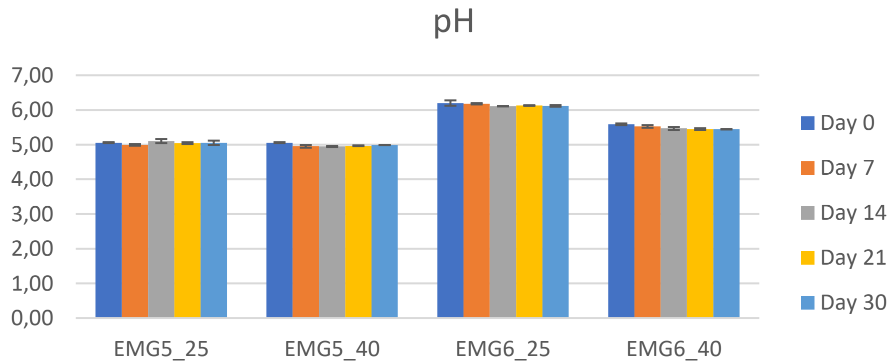


Figure 14. pH of formulations EMG5 and EMG6 over 30 days of storage at 25 °C and 40 °C.

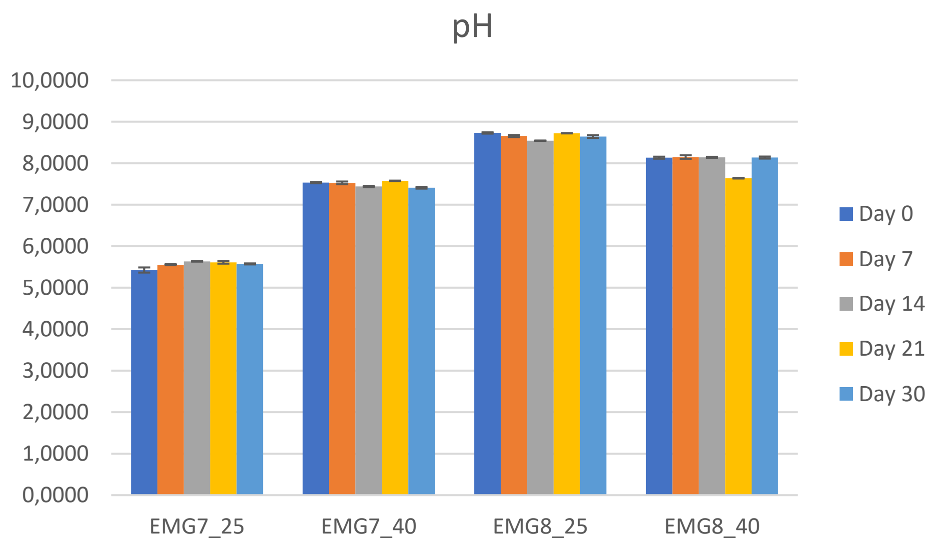


Figure 15. pH of formulations EMG7 and EMG8 over 30 days of storage at 25 °C and 40 °C.

Comparing formulations EMG5 and EMG6, it can be observed that the formulation EMG6 (with extract) had a higher pH than the formulation EMG5 (without extract), in contrast to what was observed with formulations CR2 and CR4. When comparing formulations EMG7 and EMG8, it was noticed the same behavior as seen with formulations EMG5 and EMG6. It was also observed a decrease in pH between formulations EMG5 and EMG7, which could be due to the addition of a higher percentage of Carbopol to the formulation EMG7. All the other formulations maintained a constant pH, regardless of storage conditions.

In a study conducted by Almeida *et al.* (62), a comprehensive characterization of a formulation containing *Castanea sativa* leaf extract was performed. According to the authors, the pH did not change, ranging between 4.73 and 4.76. These values are slightly different from the ones obtain in CR2, CR4, EMG5, EMG6, EMG7 and EMG8. In both studies, Carbopol and triethanolamine were

used. However, the quantities used were different, being assumed that the differences in values may be due to this fact.

In the study evaluating the physical stability in formulations with coffee silverskin the pH was also stable, without any abrupt decrease (59), varying between 4.81 and 5.82. Since the ingredients used were not the same employed in the preset study, it can be inferred that the difference in pH is due this fact.

Finally, the body formulation containing coffee silverskin and *M. sativa* extracts presented minimal pH variations, with pH ranging from 4.36 to 5.40 (60). The differences observed with the present study were probably due to Carbopol that was not used.

The results obtained in this work similar with the ones mentioned above. The differences observed can occur due to the ingredients and quantities used. Also, the differences do not seem to be due to the extract, since formulations without extract also show similar results.

4.5. Color

The measurement of color in cosmetics allows to assess the stability of the product over the time of storage. In this study, the color evaluation is employed to verify the effect of the extract on the stability of the cosmetic products developed. Therefore, the following parameters were measured: L^+ represents the light component, ranging from 0 (black) to 100 (white); a^* values give the chromatic scale between green and red; and b^* values represent the chromatic scale between blue and yellow. To calculate the Chroma parameter equation 1 was used.

Equation 1. $C^* = \sqrt{(a^2 + b^2)}$

Figures 16 to 21 illustrate the results of the color analysis through time.

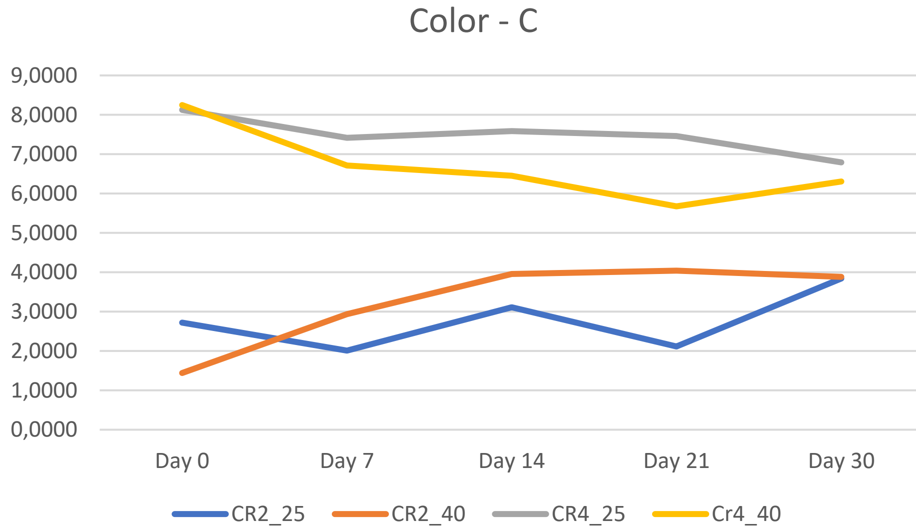


Figure 16. Comparison of Chroma parameter of formulations CR2 and CR4 over 30 days of storage at 25 °C and 40 °C.

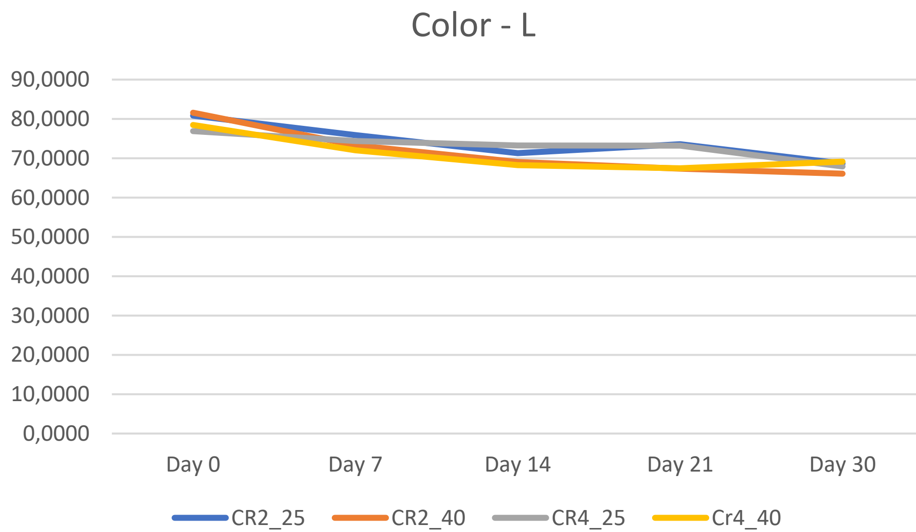


Figure 17. Comparison of L* parameter of formulations CR2 and CR4 over 30 days of storage at 25 °C and 40 °C.

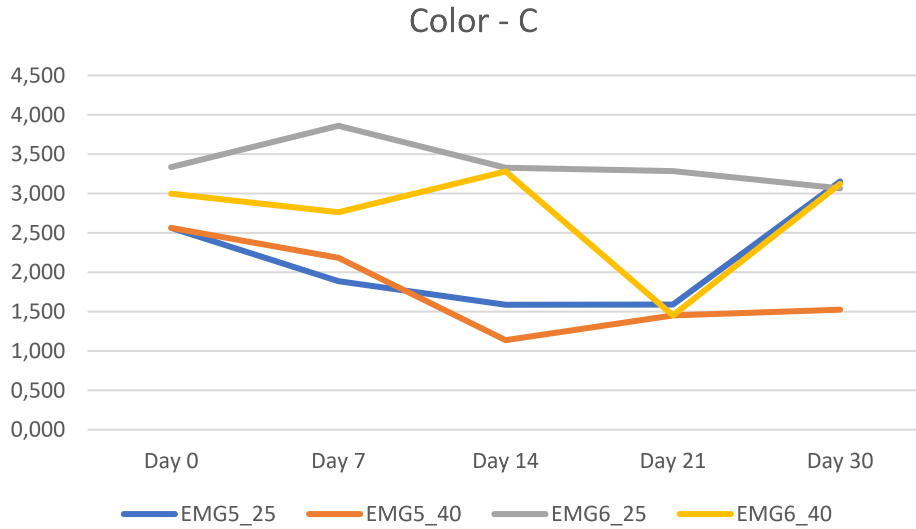


Figure 18. Comparison of Chroma parameter of formulations EMG5 and EMG6 over 30 days of storage at 25 °C and 40 °C.

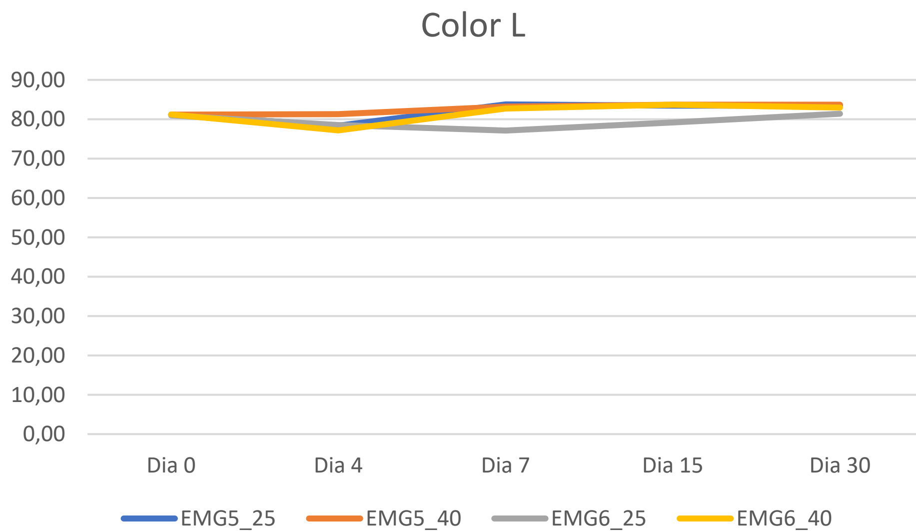


Figure 19. Comparison of L* parameter of formulations EMG5 and EMG6 over 30 days of storage at 25 °C and 40 °C.

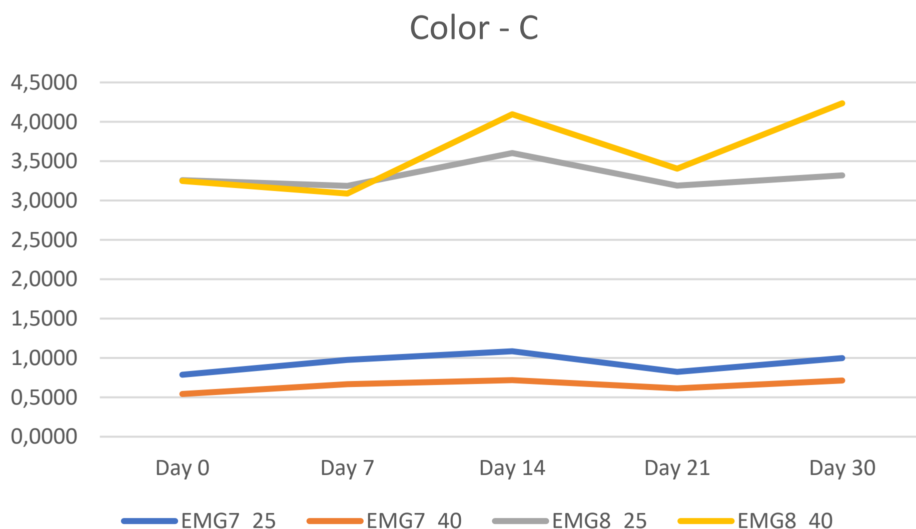


Figure 20. Comparison of Chroma parameter of formulations EMG7 and EMG8 over 30 days of storage at 25 °C and 40 °C.

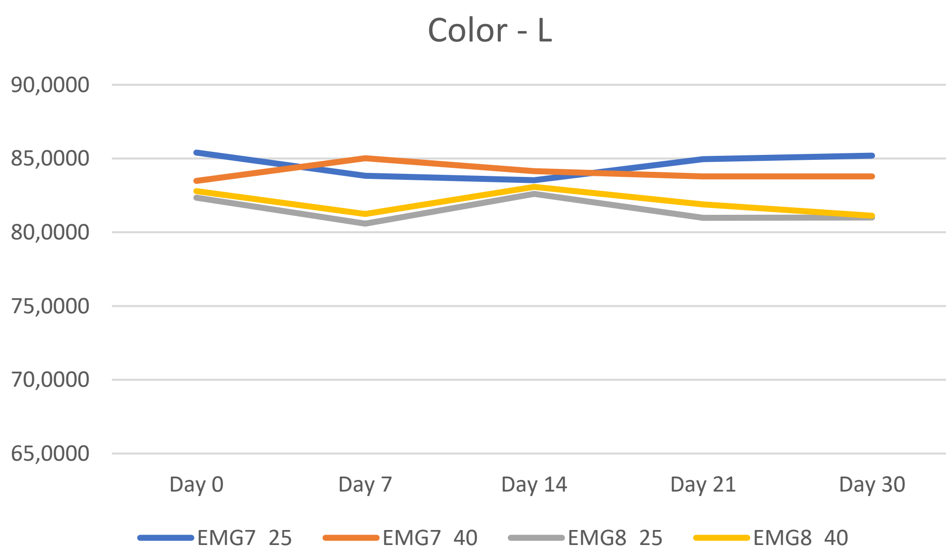


Figure 21. Comparison of L* parameter of formulations EMG7 and EMG8 over 30 days of storage at 25 °C and 40 °C.

Observing all graphs, it can be seen that the L* parameter of CR2 and CR4 formulations decreased over time, while for EMG formulations this parameter remained constant. It is also evident that the formulations with extract, stored at both temperatures, had higher values of Chroma parameter. The formulations containing the extract had a more orange hue.

It was also observed that the storage conditions did not influence the color of CR4 formulation. This formulation had much higher values of Chroma parameter than the EMG formulations containing the extract.

A cosmetic formulation containing *Fragaria vesca* leaves extract was also evaluated during stability (63). All samples were in different conditions, including at room temperature and 40 °C. According o the authors, all the samples kept at room temperature maintained the initial color, while the samples stored at 40 °C darkened over time. These results are in line with the ones achieved in the present word, with color changing from white with some orange pigment to a brown-ish white.

Pinto *et al.* (52) developed and characterized a semisolid formulation incorporating *C. sativa* shells extract. The technological stability of the formulation was evaluated over 30 days of storage at controlled temperature (25 °C). The authors compared the L* parameter of formulations with and without the extract and attested a decrease when the extract was added, from 77 to 74. The a* and b* parameter had an increase from 5.07 to 7.26 and 16.63 to 21.40, respectively. These results are also very similar to the ones described in this work, with L* values around 80. However, the a* and b* parameters are very different, with values around -2 to 0. This could be due to the more orange color of the extract.

4.6. Texture

When evaluating texture, two variables must be considered: firmness and adhesiveness. Firmness is assessed by the values of maximum force and adhesiveness is assessed by the values of the negative area of the graph force *versus* distance.

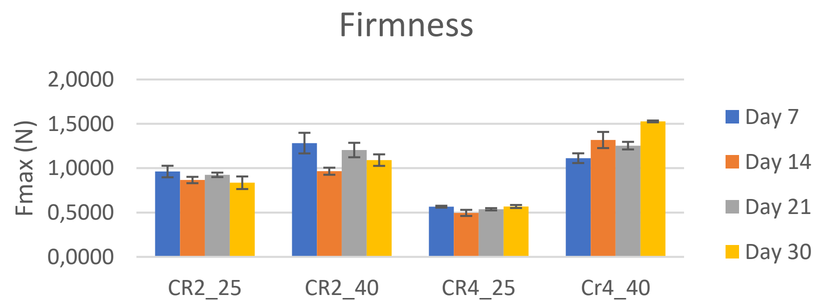
The analysis of these parameters was carried out a at two different temperatures to analyse the behaviour of the preparations when stored at room temperature and in more extreme conditions. Figure 22 shows the firmness results of formulations CR2 and CR4 over 30 days of storage at 25 °C and 40 °C. The graph indicates that the firmness at both temperatures does not present significant differences, however there is a difference between the formulations with (CR4) and without (CR2) extract, indicating that the presence of the extract caused a slight decrease in this parameter. The same pattern is noticeable when comparing formulations EMG5, EMG6 and EMG7, EMG8.

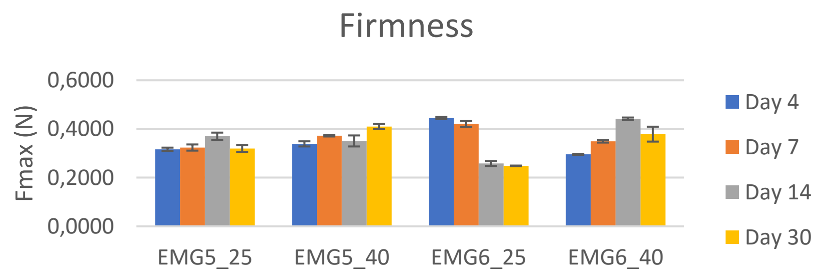
Figure 23 represent the results of adhesiveness, being possible to observe that this parameter tends to decrease over time, except for formulation EMG7 stored at 25 °C.

Comparing the values of formulations CR2 and CR4, the formulation with the extract (CR4), stored at 25 °C, had lower firmness and adhesiveness than the one without the extract (CR2).

A similar trend was seen for formulations EMG5, EMG6, EMG7 and EMG8, where the extract inclusion appears to consistently reduce the firmness and adhesiveness. However, regarding the effect of storage conditions, these results were different. In the case of the gelled multiple emulsions, the storage temperature did not affect their texture properties. This indicates that the multiple emulsion oil droplets may protect the extract, reducing its exposure to oxidation and preserving the formulation's characteristics over time.

In conclusion, the addition of the extract leads to a reduction in both firmness and adhesiveness of all formulations. However, in the case of multiple emulsions the protective effect of the oil phase seems to mitigate the impact of temperature on the texture parameters.





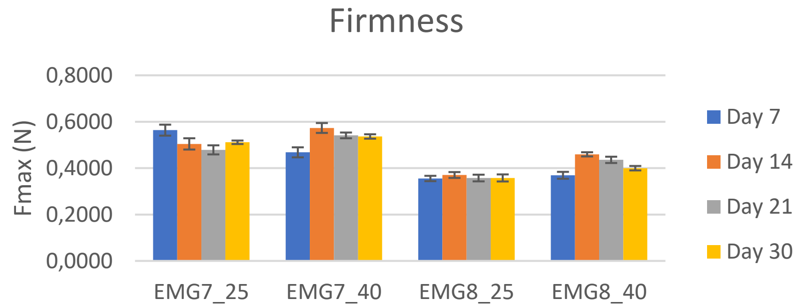
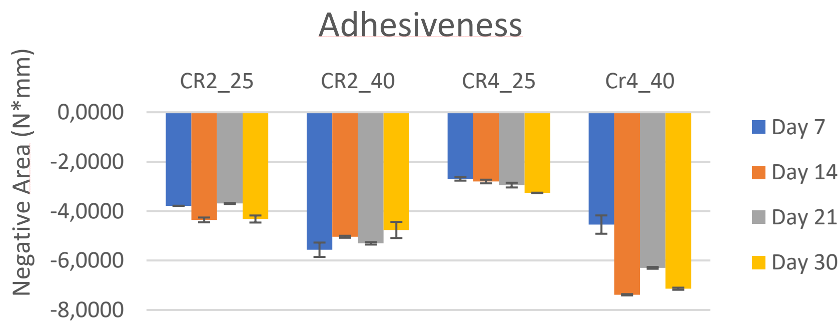
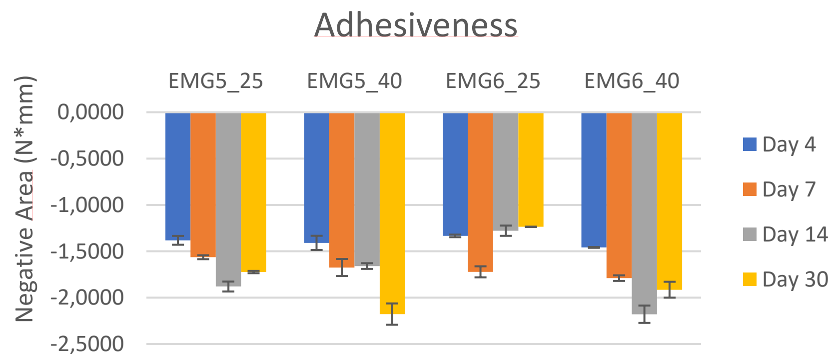


Figure 22. Firmness of formulations CR2, CR4, EMG5, EMG6, EMG7, EMG8 over 30 days of storage at 25 °C and 40 °C.





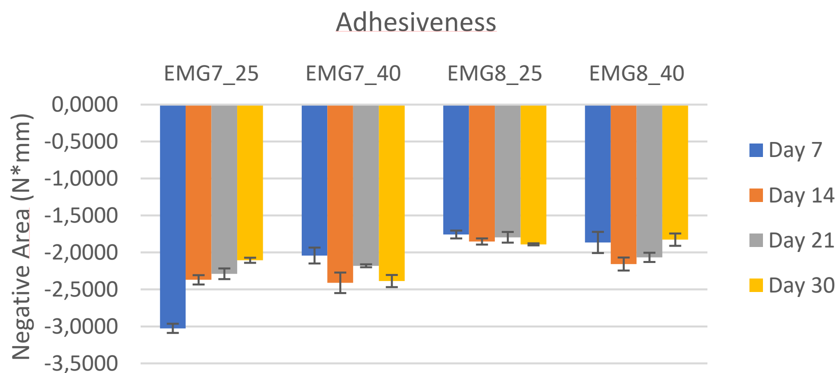


Figure 23. Adhesiveness of formulations CR2, CR4, EMG5, EMG6, EMG7, EMG8 over 30 days of storage at 25 °C and 40 °C.

Pinto *et al.* (64) developed hydrogels with *C. sativa* bur extract and compared the results with a formulation without extract. According to the authors, it was possible to conclude that adhesiveness increases with the presence of bur extract (-1.480 to -0.707). In opposite to

adhesiveness, the increase of extract concentration in gels affords the decrease of firmness (0.554 to 0.288). The same behaviour was found in the present study, however there is a difference in values, with the ones described being almost twice the ones on the paper. As the excipients used for the formulations were the same, it is expected that the difference in behaviour is due to the extract.

In another study, a semi-solid paste derived from a patent process of olive pomace extraction was evaluated regarding texture (65). The behaviour observed by the authors was similar to the present study with values changing from -0.350 to -0.300. Nonetheless, regarding firmness the same is not observed since the value increase with the extract incorporation.

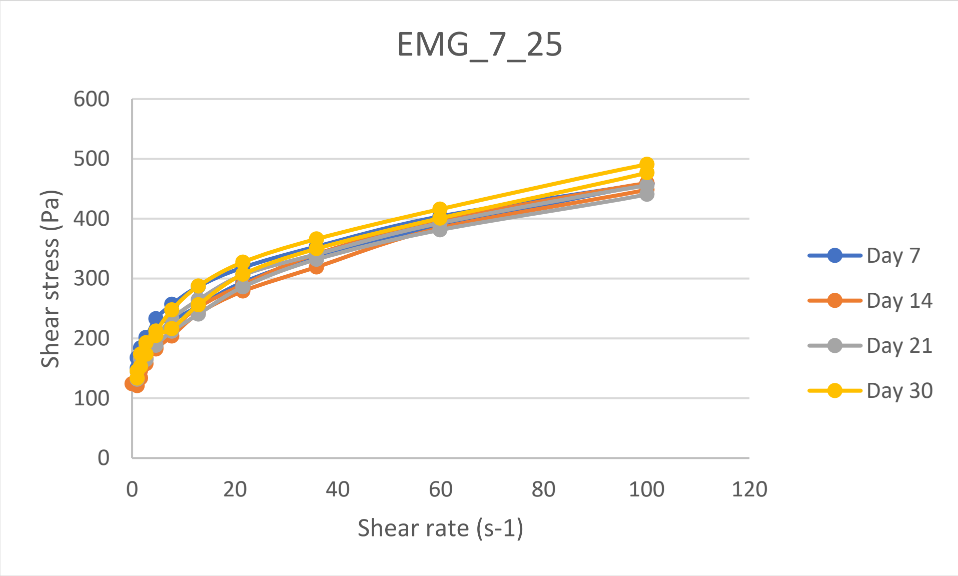
4.7. Rheologyy

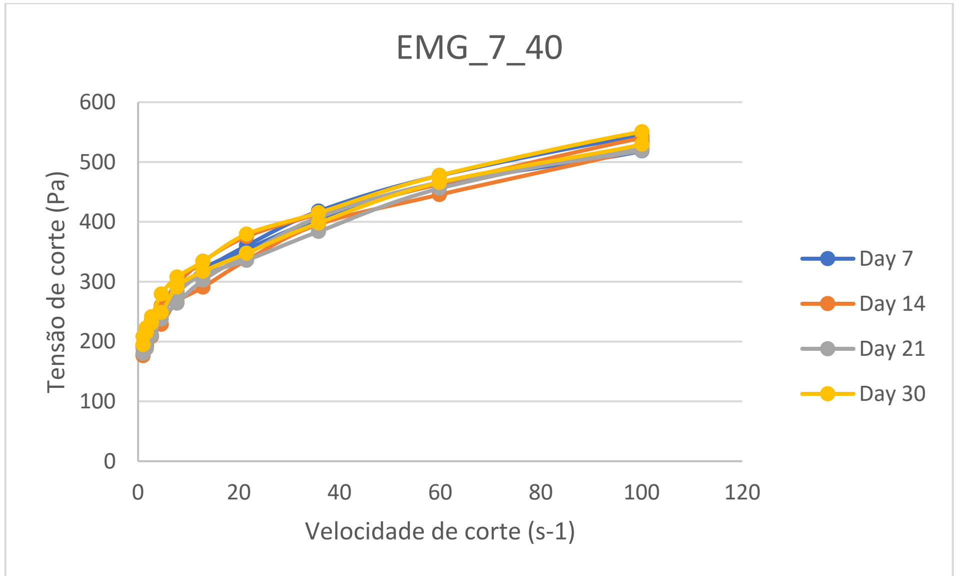
24 shows the graphs of shear stress *versus* shear rate for the formulations with and without extract stored at 25 °C and 40 °C over 30 days.

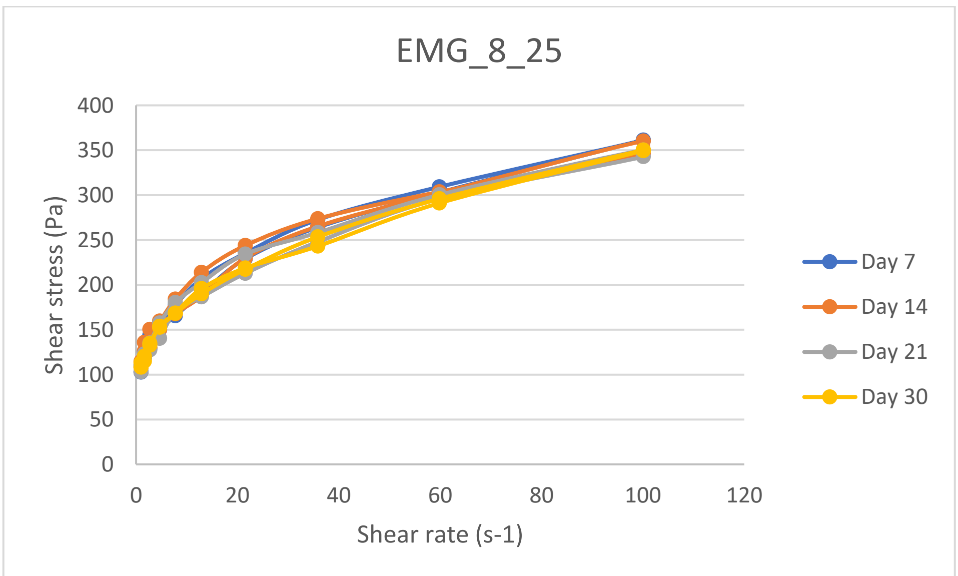
According to Figure 24, in all formulations the shear stress values at the highest shear rate vary slightly over time. However, comparing the formulations with and without extract, the formulations with the extract presented lower values. Thus, the presence of extract changes the rheological characteristics of the formulations.

All formulations presented a similar behaviour to the one presented in Figure 24. The graphics are displayed in the annexes.

Moreover, all formulations present a non-Newtonian, pseudoplastic or shear thinning behaviour with yield stress, since the apparent viscosity decreased with the increase in the shear rate, but deformation only occurred after the application of a certain shear stress. Formulations containing the extract (EMG8), stored at both temperatures, showed a slight decrease on the apparent viscosity. This behaviour is similar to the one described by Pinto *et al.* for the hydrogels prepared with *C. sativa* bur extract (64), with the formulations with extract presenting lowest values. Pinto *et al*. also described similar results in a paper with *C. sativa* shells extracts (36).


EMG_7_25
Shear stress (Pa)
600
500
400
300
200
100
0
0
20
40
60
80
100
120
Shear rate (s-1)
Day 7
Day 14
Day 21
Day 30


EMG_7_40
Tensão de corte (Pa)
600
500
400
300
200
100
0
0
20
40
60
80
100
120
Velocidade de corte (s-1)
Day 7
Day 14
Day 21
Day 30


EMG_8_25
Shear stress (Pa)
400
350
300
250
200
150
100
50
0
0
20
40
60
80
100
120
Shear rate (s-1)
Day 7
Day 14
Day 21
Day 30

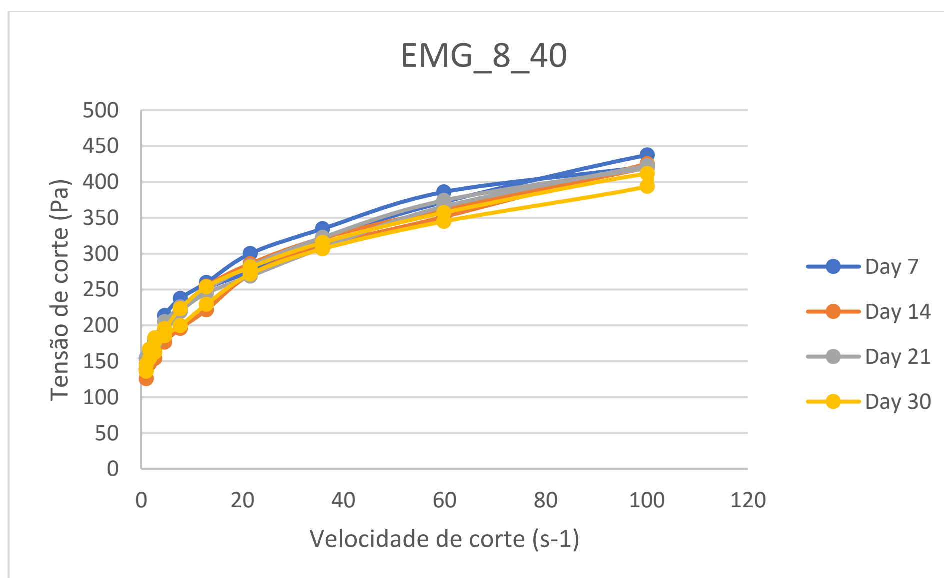


Figure 24. Rheograms of formulations EMG7 and EMG8 over 30 days of storage at 25 °C and 40 °C

4.8. Droplets size

The droplet sizes of the oil phase of the W/O/W emulsions with and without goji berries extract were evaluated using the Mastersizer 3000. The results are summarized in Table 6.

Table 6. Droplet size of the W/O/W emultions presented as mean percentile and Span value.

	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)	Span
EMG_5	0,208	0,362	0,594	1,068
EMG_6	0,217	0,372	0,605	1,042
EMG_7	0,210	0,394	0,597	1,063
EMG_8	0,211	0,365	0,598	1,061

As can be seen, all emulsions have very similar percentiles. The percentile Dx10 had a value of around 0.200 µm, the percentile Dx50 was around 0.300 µm and the percentile Dx90 was around 0.500 µm.

According to the literature, the closer the Span value is to 1, the narrower the droplet size distribution and, consequently, the more uniform the droplet size. Therefore, the lower the Span value, the more uniform the sample analyzed.

All samples studied have a Span value close to 1 and, therefore the samples are considered uniform, presenting a low dispersion of droplet sizes.

These values are within the values obtained in another study concerning the development of a solid-in-oil nanodispersion for transcutaneous delivery of proteins, in which the average droplet size obtained was between 200 and 300 nm (66).

5. Conclusions and future perspectives

Throughout this work, the importance of using natural ingredients for cosmetics was discussed. The cell viability studies performed allow to verify that goji berries are not toxic to skin.

The permeation assay provided valuable insights into the compounds that penetrate the skin, revealing that goji berries are indeed an excellent product for cosmetic application. The results demonstrated that the active ingredients present in berries have a high permeation rate, which suggests that they may offer significant benefits for skin health and appearance. This efficacy makes berries a promising option for cosmetic formulations, contributing to the development of products that enhance skin hydration, protection and rejuvenation.

Initially, a hydrophilic cream containing goji berries extract, know for its antioxidant and nourishing properties, was prepared. However, after a short period of time, it was observed that the product showed signs of oxidation, which compromised its effectiveness and potential for application. To solve this challenge, a gelled W/O/W emulsion was developed in which goji berry extract was incorporated into the innermost aqueous phase. This strategy aims to protect the extract from oxidation, ensuring that its beneficial properties are preserved and enhanced.

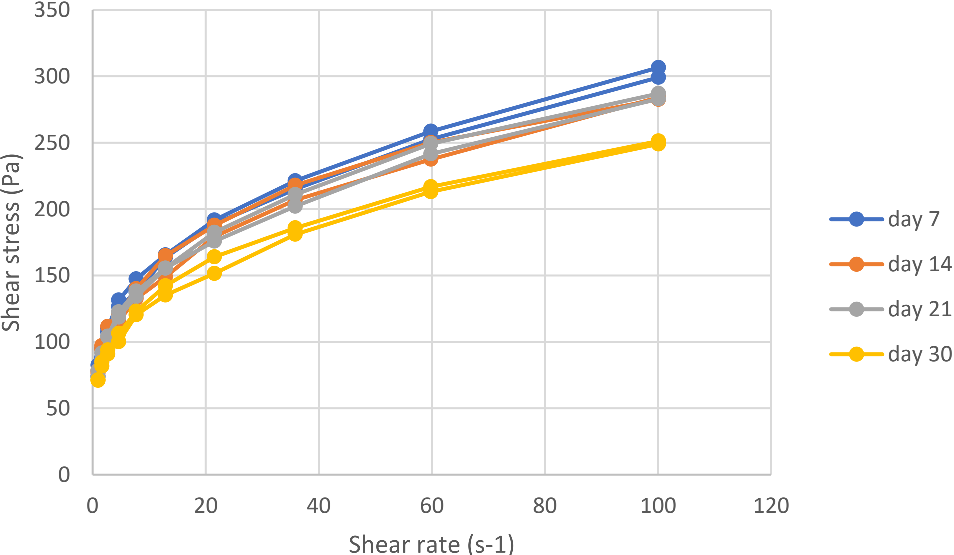
The gelled multiple emulsions with and without extract were subjected to a series of rigorous tests for characterization. The analyses included determining the droplet size of the oil phase, assessing the pH, performing accelerated stability tests and analyzing the color, rheology and texture. Th results obtained showed that these formulations presented pH values compatible with the skin, ensuring that they are safe and well tolerated during application. Furthermore, the formulations demonstrated remarkable stability over 30 days of storage, at 25°C and 40°C. The texture and rheological characteristics of the formulations were carefully evaluated and proved to be suitable for skin application, providing a possible pleasant and effective sensory experience. The stability of these properties over time reinforces the quality of the formulations developed.

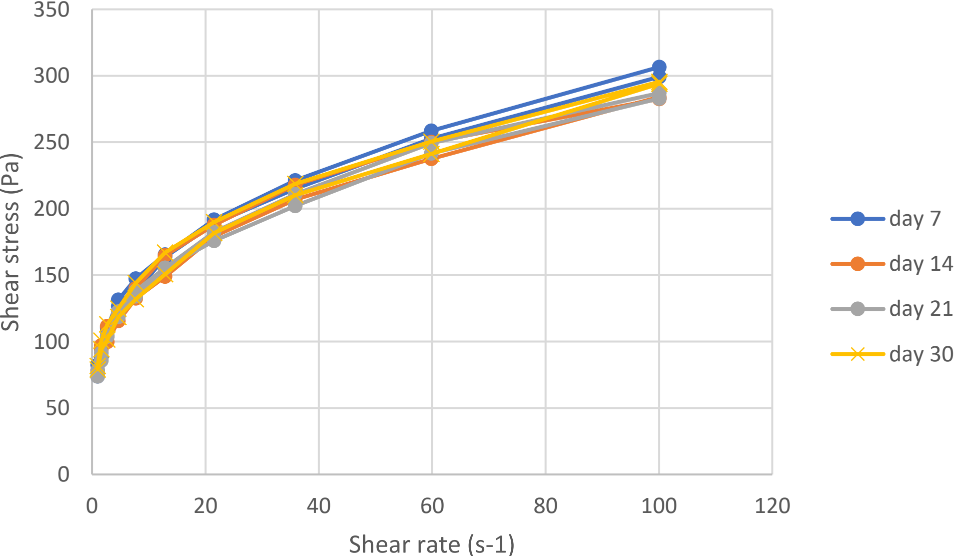
In short, the results of this work indicate that formulations containing goji berry extract have great potential to become innovative cosmetic products, especially in the prevention of skin aging. The combination of the antioxidant properties of the extract with the gelled emulsion technology not only preserves the integrity of the active ingredients, but also offers a superior user experience to the consumer.

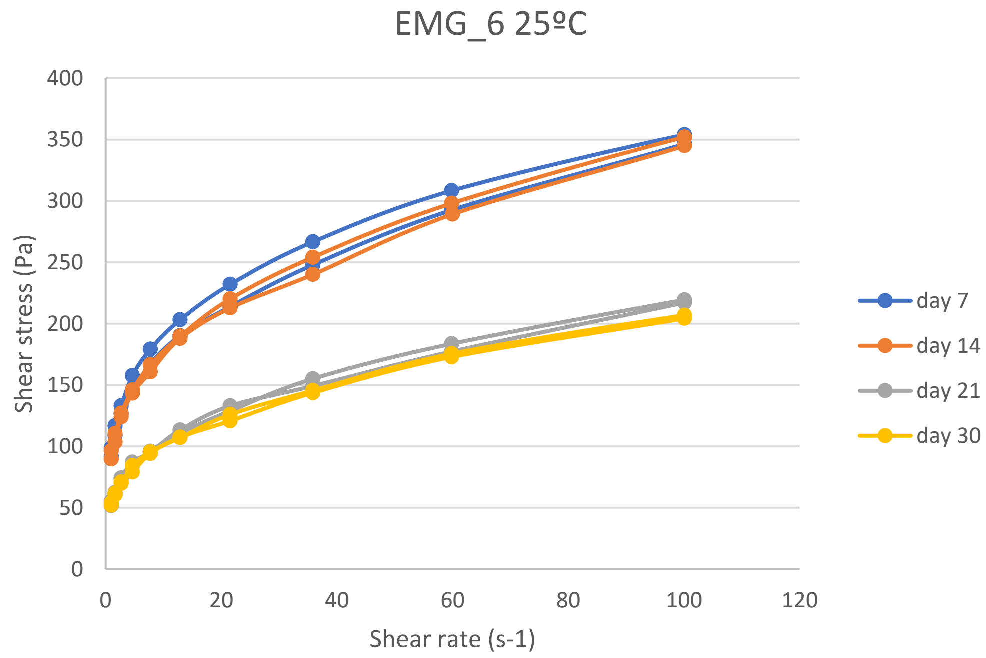
For future research, it would be highly interesting to conduct stability studies over a longer period, as well as biometric tests on human volunteers. This will allow conclusively attesting the potential

anti-aging effect of these formulations, solidifying their value in the cosmetic market. This research path promises not only to validate the results obtained, but also to open new perspectives for the development of products that promote the beauty and health of the skin in an effective and innovative way.

Annexes







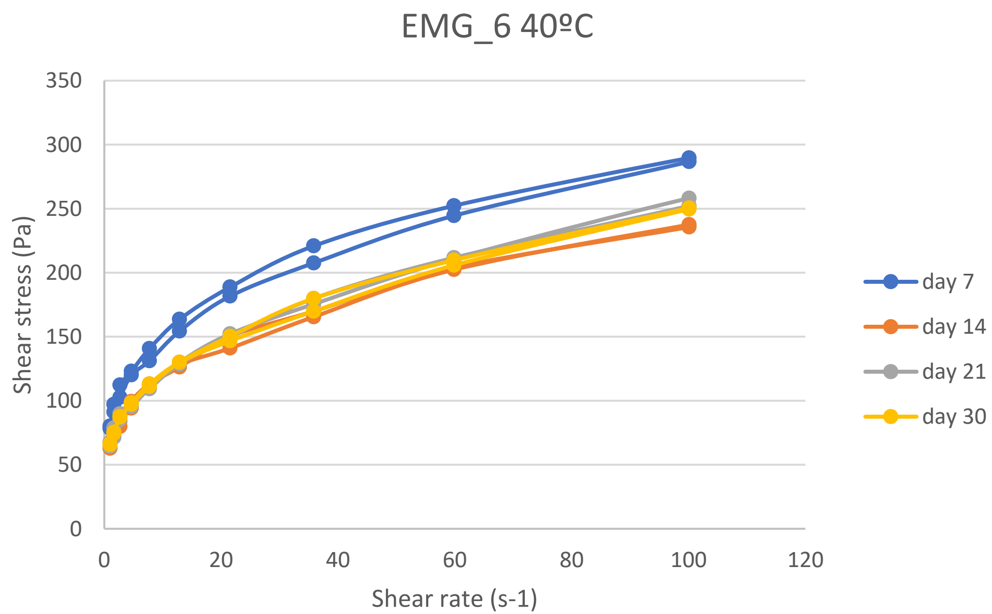


Figura 25 Rheograms of formulations EMG5 and EMG6 over 30 days of storage at 25 °C and 40 °C

References

1. Esteves SCL. Consumo de produtos à base de plantas e de plantas medicinais avaliado em espaços comerciais do distrito de Bragança. Bragança: IPB; 2015.

2. Chaiyana W, Charoensup W, Sriyab S, Punyoyai C, Neimkhum W. Herbal Extracts as Potential Antioxidant, Anti-Aging, Anti-Inflammatory, and Whitening Cosmeceutical Ingredients. Chemistry and Biodiversity. 2021

3. Rivière C, Hilbert JL. Trends in natural product research: PSE young scientists' meeting Lille 2017: Natural products in health, agro-food and cosmetics. Phytochemistry Reviews. 2018;17(5):947–9.

4. Hunt KJ, Hung SK, Ernst E. Botanical Extracts as Anti-Aging Preparations for the Skin: A Systematic Review. Drugs & Aging. 2010;27(12):973–85.

5. McMullen RL, Dell'Acqua G. History of Natural Ingredients in Cosmetics. Cosmetics. 2023;10(3):71.

6. Nadeeshani Dilhara Gamage DG, Dharmadasa RM, Chandana Abeysinghe D, Saman Wijesekara RG, Prathapasinghe GA, Someya T. Global Perspective of Plant-Based Cosmetic Industry and Possible Contribution of Sri Lanka to the Development of Herbal Cosmetics. Rahman K,editor. Evidence-Based Complementary Alternative Medicine. 2022;2022:1–26.

7. Jatoi MA, Fruk M, Buhin J, Vinceković M, Vuković M, Jemrić T. Effect of Different Storage Temperatures on Storage Life, Physico-chemical and Sensory Attributes of Goji Berry (Lycium barbarum L.) Fruits. Erwerbs-Obstbau. 2018;60(2):119–26.

8. Tian S, Yu Y, Liu Q, Guo H, Yu J, Wang X, et al. An integrated strategy for the geographical origin traceability of Goji berries by antioxidants characteristic fingerprint based online ultra-performance liquid chromatography-2,2-diphenyl-1-picrylhydrazyl- photodiode array detector-mass spectrometry combined with multivariate statistics analysis. Jounal of Separation Science. 2023;46(7):2200826.

9. Ma ZF, Zhang H, Teh SS, Wang CW, Zhang Y, Hayford F, et al. Goji Berries as a Potential Natural Antioxidant Medicine: An Insight into Their Molecular Mechanisms of Action. Oxidativa Medicine and Cellular Longevity; 2019;2019:1–9.

10. Yu J, Yan Y, Zhang L, Mi J, Yu L, Zhang F, et al. A comprehensive review of goji berry processing and utilization. Food Science & Nutrition 2023;11(12):7445–57.

11. Lu Y, Guo S, Zhang F, Yan H, Qian D wei, Shang E xin, et al. Nutritional components characterization of Goji berries from different regions in China. Journal of Pharmaceutical and Biomedical Analysis. 2021;195:113859.

12. Zhao WH, Shi YP. Comprehensive analysis of phenolic compounds in four varieties of goji berries at different ripening stages by UPLC–MS/MS. Journal of Food Composition and Analysis. 2022;106:104279.

13. Povolo C, Foschini A, Ribaudo G. Optimization of the extraction of bioactive molecules from *Lycium barbarum* fruits and evaluation of the antioxidant activity: a combined study. Natural Product Research. 2019;33(18):2694–8.

14. Vidović BB, Milinčić DD, Marčetić MD, Djuriš JD, Ilić TD, Kostić AŽ, et al. Health Benefits and Applications of Goji Berries in Functional Food Products Development: A Review. Antioxidants. 2022;11(2):248.

15. Teixeira F, Silva AM, Delerue-Matos C, Rodrigues F. Lycium barbarum Berries (Solanaceae) as Source of Bioactive Compounds for Healthy Purposes: A Review. International Journal of Molecular Sciences. 2023;24(5):4777.

16. Ilić T, Dodevska M, Marčetić M, Božić D, Kodranov I, Vidović B. Chemical Characterization, Antioxidant and Antimicrobial Properties of Goji Berries Cultivated in Serbia. Foods. 2020;9(11):1614.

17. Ducruet J, Rébénaque P, Diserens S, Kosińska-Cagnazzo A, Héritier I, Andlauer W. Amber ale beer enriched with goji berries – The effect on bioactive compound content and sensorial properties. Food Chemistry. 2017;226:109–18.

18. Liu J, Meng J, Du J, Liu X, Pu Q, Di D, et al. Preparative Separation of Flavonoids from Goji Berries by Mixed-Mode Macroporous Adsorption Resins and Effect on Aβ-Expressing and Anti-Aging Genes. Molecules. 2020;25(15):3511.

19. Potterat O. Goji (*Lycium barbarum* and *L. chinense*): Phytochemistry, Pharmacology and Safety in the Perspective of Traditional Uses and Recent Popularity. Planta Medica. 2010;76(01):7–19.

20. Fatchurrahman D, Amodio ML, Colelli G. Quality of Goji Berry Fruit (Lycium barbarum L.) Stored at Different Temperatures. Foods. 2022;11(22):3700.

21. Teodoro AJ. Bioactive Compounds of Food: Their Role in the Prevention and Treatment of Diseases. Oxidative Medicine and Cellular Longevity. 2019;2019:1–4.

22. Ceccarini MR, Codini M, Cataldi S, Fioretti B, Brecchia G, Albi E, et al. Licium Barbarum cultivated in Italy: Chemical characterization and nutritional evaluation. Italian Journal of Food Science. 2022;34(3):59–65.

23. Zia-Ul-Haq M, Dewanjee S, Riaz M, editores. Carotenoids: Structure and Function in the Human Body. Cham: Springer International Publishing; 2021

24. Liu JK. Natural products in cosmetics. Natural Products and Bioprospecting. 2022;12(1):40.

25. Ma'or Z, Cohen D, La'or-Costa Y, Portugal-Cohen M. Safe Retinol-Like Skin Biological Effect by a New Complex, Enriched with Retinol Precursors. Journal of Cosmetics, Dermatological Sciences and Applications. 2020;10(02):59–75.

26. Md. Mamunur Rashid, Shariful Islam, Md. Nazim Uddin, Md. Zia Uddin Al Mamun, Md. Jaynal Abedin, Md. Habibur Rahman Bhuiyan, Md. Abdus Satter Miah,. HPLC-DAD analysis of water-soluble vitamins (B1, B2, B3, B5, B6, C and Biotin) and fat-soluble vitamins (A, D, E, K1 and β-

carotene) in commonly consumed pulses in Bangladesh, Applied Food Research. 2024;Volume 4(1).

27. Luo Q, Cai Y, Yan J, Sun M, Corke H. Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from Lycium barbarum. Life Sciences. 2004;76(2):137–49.

28. Kulczyński B, Gramza-Michałowska A. Goji Berry (Lycium barbarum): Composition and Health Effects – a Review. Polish Journal of Food and Nutrition Sciences. 2016; 66(2):67–75.

29. Sami A, Han S, Haider MZ, Khizar R, Ali Q, Shafiq M, et al. Genetics aspect of vitamin C (Ascorbic Acid) biosynthesis and signaling pathways in fruits and vegetables crops. Functional & Integrative Genomics. 2024;24(2):73.

30. Armenta S, Garrigues S, Esteve-Turrillas FA, De La Guardia M. Green extraction techniques in green analytical chemistry. TrAC Trends in Analytical Chemistry. 2019;116:248–53.

31. Rodríguez García SL, Raghavan V. Green extraction techniques from fruit and vegetable waste to obtain bioactive compounds—A review. Critical Reviews in Food Science and Nutrition. 2022;62(23):6446–66.

32. Panja P. Green extraction methods of food polyphenols from vegetable materials. Current Opinion in Food Science. 2018;23:173–82.

33. Skenderidis P, Petrotos K, Giavasis I, Hadjichristodoulou C, Tsakalof A. Optimization of ultrasound assisted extraction of of goji berry (*Lycium barbarum*) fruits and evaluation of extracts' bioactivity. Journal of Food Process Engineering. 2017;40(5):e12522.

34. Usman M, Nakagawa M, Cheng S. Emerging Trends in Green Extraction Techniques for Bioactive Natural Products. Processes. 2023;11(12):3444.

35. Kumar K, Srivastav S, Sharanagat VS. Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. Ultrasonics Sonochemistry. 2021;70:105325.

36. Pinto D, Cádiz-Gurrea MDLL, Vallverdú-Queralt A, Delerue-Matos C, Rodrigues F. Castanea sativa shells: A review on phytochemical composition, bioactivity and waste management approaches for industrial valorization. Food Research International. 2021;144:110364.

37. Dzah CS, Duan Y, Zhang H, Wen C, Zhang J, Chen G, et al. The effects of ultrasound assisted extraction on yield, antioxidant, anticancer and antimicrobial activity of polyphenol extracts: A review. Food Bioscience. 2020;35:100547.

38. Silva AM, Pinto D, Moreira MM, Costa PC, Delerue-Matos C, Rodrigues F. Valorization of Kiwiberry Leaves Recovered by Ultrasound-Assisted Extraction for Skin Application: A Response Surface Methodology Approach. Antioxidants. 2022;11(4):763.

39. Barthe M, Bavoux C, Finot F, Mouche I, Cuceu-Petrenci C, Forreryd A, et al. Safety Testing of Cosmetic Products: Overview of Established Methods and New Approach Methodologies (NAMs). Cosmetics. 2021;8(2):50.

40. Almeida A, Sarmento B, Rodrigues F. Insights on in vitro models for safety and toxicity assessment of cosmetic ingredients. International Journal of Pharmaceutics 2017;519(1–2):178–85.

41. Pinto D, Cádiz-Gurrea MDLL, Garcia J, Saavedra MJ, Freitas V, Costa P, et al. From soil to cosmetic industry: Validation of a new cosmetic ingredient extracted from chestnut shells. Sustainable Materials and Technologies. 2021;29:e00309.

42. Su Z, Luo F ya, Pei X rong, Zhang F lan, Xing S xia, Wang G li. Final Publication of the "Regulations on the Supervision and Administration of Cosmetics" and New Prospectives of Cosmetic Science in China. Cosmetics. 2020;7(4):98.

43. Pauwels M. Safety evaluation of cosmetics in the EUReality and challenges for the toxicologist. Toxicol Lett. 2004

44. Rodrigues F, Pereira C, Pimentel FB, Alves RC, Ferreira M, Sarmento B, et al. Are coffee silverskin extracts safe for topical use? An in vitro and in vivo approach. Industrial Crops and Products. 2015;63:167–74.

45. Dent M, Amaral RT, Da Silva PA, Ansell J, Boisleve F, Hatao M, et al. Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients. Computational Toxicology. 2018;7:20–6.

46. Fonseca-Santos B, Corrêa MA, Chorilli M. Sustainability, natural and organic cosmetics: consumer, products, efficacy, toxicological and regulatory considerations. Brazilian Journal of Pharmaceutical Sciences. 2015;51(1):17–26.

47. NGUYEN PND, NGUYEN VT, VO NNT. Key Determinants of Repurchase Intention toward Organic Cosmetics. The Journal of Asian Finance, Economics and Business. 2019;6(3):205–14.

48. Lee J, Kwon KH. Future perspective safe cosmetics: Focused on associated with ISO natural organic index. Journal of Cosmetic Dermatology. 2022;21(12):6619–27.

49. Dahal RH, Shim DS, Kim J. Development of actinobacterial resources for functional cosmetics. Journal of Cosmetic Dermatology. 2017;16(2):243–52.

50. Martins AM, Marto JM. A sustainable life cycle for cosmetics: From design and development to post-use phase. Sustainable Chemistry and Pharmacy, 2023;35:101178.

51. Rocca R, Acerbi F, Fumagalli L, Taisch M. Sustainability paradigm in the cosmetics industry: State of the art. Cleaner Waste Systems. 2022;3:100057.

52. Pinto D, Lameirão F, Delerue-Matos C, Rodrigues F, Costa P. Characterization and Stability of a Formulation Containing Antioxidants-Enriched Castanea sativa Shells Extract. Cosmetics. 2021;8(2):49.

53. Rábová TK. Marketing Communication of SMEs Specialized in Cosmetic Industry in Magazines for Women. Procedia - Social and Behavioral Sciences. 2015;175:48–57.

54. Venciute D, Kazukauskaite M, Correia RF, Kuslys M, Vaiciukynas E. The effect of cause-related marketing on the green consumption attitude–behaviour gap in the cosmetics industry. Journal of Contemporary Marketing Science. 2023;6(1):22–45.

55. Teixeira F, Silva AM, Sut S, Dall'Acqua S, Ramos OL, Ribeiro AB, et al. Ultrasound-assisted extraction of bioactive compounds from goji berries: Optimization, bioactivity, and intestinal permeability assessment. Food Research International. 2024;188:114502.

56. Silva AM, Pinto D, Fernandes I, Gonçalves Albuquerque T, Costa HS, Freitas V, et al. Infusions and decoctions of dehydrated fruits of Actinidia arguta and Actinidia deliciosa: Bioactivity, radical scavenging activity and effects on cells viability. Food Chemistry. 2019;289:625–34.

57. Silva AM, Garcia J, Dall'Acqua S, Costa P, Delerue-Matos C, Rodrigues F. Eco-friendly insights on kiwiberry leaves valorization through in-vitro and in-vivo studies. Industrial Crops and Products. 2022;184:115090.

58. Taofiq O, Rodrigues F, Barros L, Barreiro MF, Ferreira ICFR, Oliveira MBPP. Mushroom ethanolic extracts as cosmeceuticals ingredients: Safety and ex vivo skin permeation studies. Food and Chemical Toxicology. 2019;127:228–36.

59. Rodrigues F, Gaspar C, Palmeira-de-Oliveira A, Sarmento B, Helena Amaral M, P. P. Oliveira MB. Application of Coffee Silverskin in cosmetic formulations: physical/antioxidant stability studies and cytotoxicity effects. Drug Development and Industrial Pharmacy. 2016;42(1):99–106.

60. Rodrigues F, Sarmento B, Amaral MH, Oliveira MBPP. Exploring the antioxidant potentiality of two food by-products into a topical cream: stability, *in vitro* and *in viv*o evaluation. Drug Development and Industrial Pharmacy. 2016;42(6):880–9.

61. Santos ML. Desenvolvimentos de nanossistemas oara promocção da penetração cutânea da cafeína. Porto: Faculdade de farmácia da Universidade do Porto; 2022.

62. Almeida IF, Maleckova J, Saffi R, Monteiro H, Góios F, Amaral MH, et al. Characterization of an antioxidant surfactant-free topical formulation containing *Castanea sativa* leaf extract. Drug Development and Industrial Pharmacy. 2015;41(1):148–55.

63. Couto J, Figueirinha A, Batista MT, Paranhos A, Nunes C, Gonçalves LM, et al. Fragaria vesca L. Extract: A Promising Cosmetic Ingredient with Antioxidant Properties. Antioxidants. 2020;9(2):154.

64. Pinto D, Braga N, Rodrigues F, Oliveira M. Castanea sativa Bur: An Undervalued By-Product but a Promising Cosmetic Ingredient. Cosmetics. 2017;4(4):50.

65. Rodrigues R, Lobo JC, Ferreira DM, Senderowicz E, Nunes MA, Amaral MH, et al. Chemical and Rheological Characterization of a Facial Mask Containing an Olive Pomace Fraction. Cosmetics. 2023;10(2):64.

66. Tahara Y, Honda S, Kamiya N, Piao H, Hirata A, Hayakawa E, et al. A solid-in-oil nanodispersion for transcutaneous protein delivery. Journal of Controlled Release. 2008;131(1):14–8.