

Article

Characterization and Stability of a Formulation Containing Antioxidants-Enriched *Castanea sativa* Shells Extract

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Abstract: The cosmetic industry is a field on rise where the search for novel antioxidant ingredients, preferably from natural matrixes, became a new challenge. The reuse of *Castanea sativa* shells as a source of phytochemicals with pro-healthy effects in skin care products may be a valuable alternative to valorize this underexploited agro-industrial by-product. A previous study of our research group demonstrated the antioxidant properties of chestnut shells extract obtained by ultrasound-assisted extraction (UAE), as well as its safeness on skin cell lines, namely keratinocytes and fibroblasts. Based on the extract richness in antioxidants, a formulation containing *C. sativa* shells extract obtained by this environmentally friendly technology was successfully developed in the present study. The oil-in-water (O/W) cream incorporating the chestnut shells extract was further characterized regarding organoleptic and technological properties, namely color, pH, droplets size, and viscosity. A stability study over time was also accomplished. The results demonstrate that the formulation not only displayed pleasant organoleptic characteristics attractive to consumers but also a pH compatible with skin and a suitable viscosity for topical application. The stability study revealed minor changes to droplet size and color, without affecting the general stability of the formulation. In conclusion, this study emphasized the prominent benefits of chestnut shells extract as a novel ingredient for skin care formulations.

Keywords: chestnut shells; antioxidants; ultrasound-assisted extraction; oil-in-water formulation; technological properties; stability



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1. Introduction

Skin aging is a complex and multifactorial biological process that results from the cumulative effects of intrinsic (e.g., genetic predisposition, hormonal disorders, and vitamin deficiencies) and extrinsic factors (e.g., ultraviolet (UV) radiation, environmental pollution, and improper skin care), which lead to gradual damages to skin integrity and physiological functions [1,2]. The major cause of skin aging is chronic exposure to UV rays, inducing skin harmful effects and prompting oxidative damages to DNA, lipids, and proteins via the overproduction of free radicals [1,2]. Characteristic signs of skin aging include the loss of skin elasticity and wrinkling due to decreased levels of collagen production and rapid collagen breakdown by matrix metalloproteinases (MMPs) [2,3]. Besides endogenous antioxidants present in human skin (e.g., glutathione, superoxide dismutase, catalase), exogenous antioxidants, normally carried by topical formulations, may also exert a key role in mitigating the biochemical consequences of oxidative stress by preventing protein and lipid oxidation, enhancing DNA repair, and scavenging free radicals [2,3]. Considering skin aging, antioxidants provide protection from sunburns and photoaging, stimulate skin

repair, hinder pigmentation, and increase skin hydration [1]. The demand for new cosmetic agents mostly derived from botanicals has grown, mainly attracted by the anti-aging effects reported, the ability to counteract skin aging signs and enhance the oxidative stability of oil-in-water (O/W) emulsions [3]. On the other hand, the employment of environmentally friendly extraction technologies, such as ultrasound-assisted extraction (UAE), also contributes to sustainable practices, allowing the isolation of bioactive compounds from agro-residues [4].

Currently, consumerism has attained the cosmetic industry. The Cosmetics Europe The Personal Care Association reported that 450 million Europeans daily use a wide variety of cosmetic products, which explains the environmental impacts of skin care companies, particularly due to unsustainable sourcing of raw materials, pollution during manufacturing, and the disposal of packaging and the remaining products [2,5]. Hence, cosmetic companies have attempted to create greener formulations using sustainable raw materials. For a long time, natural compounds (such as phytonutrients, microbial metabolites, minerals, and animal protein components) have been generally considered beneficial for healthy skin aging, and are already employed in cosmetics marketed on a large scale [2,6]. Considering sustainability concerns, the valorization of underexploited food waste answers to different Sustainable Development Goals as defined by the United Nations' Agenda 2030, and simultaneously provides new ways to recover valuable compounds aimed at a cosmetic application [4,7]. Nevertheless, European Regulation No. 1223/2009 establishes the requirements for cosmetic products marketed within the European Union and reinforces the importance of attesting to the safety of the ingredients and final products prior to commercialization. Some relevant information about the cosmetic product under assessment should be provided, namely the qualitative and quantitative composition, the physicochemical characteristics, the stability, the microbiological quality, the presence of impurities or contaminants (e.g., heavy metals, forbidden substances), the adverse reactions after exposure to the product and/or its ingredients, and the toxicological profile of the ingredients used. A final conclusion about the product's safety should be also provided and justified based on the scientific evidence. Considering the physicochemical characteristics, a semisolid formulation must have a pH compatible with skin (4–6) and appropriate viscosity. The color and rheological properties are extremely important parameters for consumer acceptance since consumers usually prefer formulations with warm colors, mild odors, and that are easy to spread on the skin [2,3]. Furthermore, the European Regulation highlights that the claims of a cosmetic product must be supported by scientific evidence. In the case of an antioxidants-enriched formulation, the antioxidant activity needs to be proven by experimental outcomes, and the antioxidant compounds that contribute to its bioactivity should be identified.

Castanea sativa Mill. (Fagaceae family) is a highly appreciated nut in Mediterranean countries due to its unique sensorial characteristics, nutritional value, and phytochemical composition [4]. The chestnut farming and processing industry generates huge amounts of by-products, most of which are shells that represent almost 20% of the total fruit weight [8]. Different authors have encouraged the reuse of chestnut shells in the cosmetic field as a promising source of added value compounds, mainly polyphenols (phenolic acids and ellagitannins), vitamin E, and amino acids, which provide antimicrobial, antioxidant, and anti-inflammatory properties as well as other beneficial skin effects, namely skin repairing, protection against free radical-mediated injuries, and strengthening of the immune system [8–13]. According to Squillaci et al. and Ham et al., shells are effective sources of natural antioxidants and deodorant compounds [12,14]. For instance, Squillaci et al. reported the protective effects of chestnut shells hydro-alcoholic extract against collagen degradation and upgrading skin hydration [12]. Beyond the antioxidant and chemoprotective abilities, chestnut shells also showed low toxicity and few side effects for dermal cells [12,15]. Nevertheless, the incorporation of chestnut shells extracts as an active ingredient in cosmetic formulations has not yet been demonstrated, forming an opening field of research. In our recent study, the extraction of chestnut shells by UAE was optimized using a math-

emational tool (Response Surface Methodology) [15]. The optimal extract was achieved at the binomial 70 °C/40 min, revealing high amounts of ellagic acid (40.4 µg/mg dw), caffeic acid derivative (15.4 µg/mg dw), and epigallocatechin (15.3 µg/mg dw), as well as remarkable in vitro scavenging capacity, particularly against nitric oxide and hypochlorous acid ($IC_{50} = 0.1 \mu\text{g/mL}$ and $IC_{50} = 0.7 \mu\text{g/mL}$, respectively). It is worth noting the potential safeness of the extract up to 100 µg/mL was proved on two skin cell lines (HFF-1 and HaCaT) [15]. These outcomes allow us to obtain *C. sativa* shells extracts with interesting pro-healthy effects to be incorporated into cosmetic products.

The aim of the present study was to develop and characterize a semisolid formulation (O/W cream) incorporating *C. sativa* shells extract obtained by a green technology (UAE), proposing a cosmetic use for this agro-industrial by-product. Additionally, the technological stability of the formulation was evaluated over 30 days of storage at controlled temperature ($25 \pm 1 \text{ }^{\circ}\text{C}$). To the best of our knowledge, this is the first study that employed chestnut shells extract as a novel cosmetic ingredient in the designing of an added value skin care product.

2. Materials and Methods

2.1. Chemicals and Reagents

All chemicals and solvents used were of analytical grade, used as received or dried by standard procedures, and obtained from commercial sources. Methanol was supplied by Honeywell (Seelze, Germany), while rutin was purchased from Sigma-Aldrich (Steinheim, Germany). Ultrapure water was obtained from a water purification system (Direct-Q Ultrapure Water Systems, Merck Millipore, Germany).

For the preparation of cosmetic formulations, Lanette N (batch No. 00338149) and Cetiol V[®] (batch No. LC-216) were obtained from José M. Vaz Pereira, S.A., while white petrolatum (batch No. 161740), glycerin (batch No. 171471), and methylparaben (batch No. 180545-J-3) were provided by Acofarma.

2.2. *Castanea sativa* Shells

Chestnut shells, kindly provided by Sortegel (Sortes, Bragança, Portugal) in October 2018, were dehydrated (Excalibur Food Dehydrator, USA) at 40 °C for 24 h and grounded to a particle size of 1 mm using an ultra-centrifugal grinder ZM200 (Retsch, Germany). Afterwards, the shells were thoroughly mixed and stored at room temperature in the dark until further extraction.

2.3. Preparation of *C. sativa* Shells Extract by Ultrasound-Assisted Extraction

The UAE was accomplished as described by Lameirão et al. [15], using an ultrasonic probe system (Sonic Vibracell, model VC 750, Newtown, CT, USA) assembled with a 13 mm diameter tip containing an amplitude, temperature, and time controller. Briefly, the powdered shells (5 g) were extracted with water (100 mL) in the ultrasonic device at 70 °C for 40 min. The amplitude and frequency were defined at 50% and 20 KHz, respectively. The extraction conditions employed were selected based on a previous study published by our research group that optimized the recovery of chestnut shells' bioactive compounds by UAE using a response surface methodology [15]. Afterwards, the extract was filtered through Whatman No. 1 paper and centrifuged (Sigma 3-30KS, Sigma, Osterode am Harz, Germany) at $16,000 \times g$ for 10 min. Then, the extract was stored at 4 °C until incorporation in the cosmetic formulation. The UAE achieved a yield of $16.1 \pm 2.5\%$, as reported in our previous paper [15]. A total of 3 batches of the extract were prepared.

2.4. Quantification of Rutin in *C. sativa* Shells Extract by HPLC-UV-Vis

The quantification of rutin was performed using a chromatographic methodology by HPLC-UV-Vis. Rutin was the only phenolic compound quantified, as it is the most active phenolic compound present in chestnut shells in terms of cosmetic use [15]. First, an absorption spectrum (absorbance versus wavelength) was obtained using a UV-Vis

spectrophotometer (Jasco, V-650, Japan) to record the maximum and minimum absorption of rutin. A rutin solution of 1 mg/mL was prepared in methanol and the absorbance was subsequently measured in a quartz cuvette with a 1 cm optical path.

For the chromatographic analysis, an HPLC system (Ultimate 3000, Thermo Scientific, UK) equipped with an autosampler, a four-phase pump, and a multichannel UV-Vis detector was used. An Acclaim™ 120 C18 column (100 mm length, 4.6 mm diameter, and a particle size of 5 µm) set at 20 °C was employed. A total of 6 standard rutin solutions (1.36–10.08 µg/mL) were prepared to plot the calibration curve. The lyophilized extract (15 mg) was dissolved in methanol (5 mL). The solution was then filtered using a 0.45 µm pore hydrophobic PTFE syringe filter with a 13 mm diameter (FilterBio, Jiangsu, China) and transferred into injection vials. The same procedure was applied to the liquid extract stored at 4 °C. The extracts were analyzed in triplicate. The injection volume was 20 µL and the flow rate was 1.5 mL/min. Each analysis was carried out for 18 min. The solvents were (A) H₂O/CH₃COOH with pH 3.0 (98:2, v/v) and (B) CH₃COOH/CH₃CN/H₂O (2:20:78, v/v/v). The gradient consisted of 90% eluent (A) and 10% eluent (B) in the first 14 min; 70% eluent (A) and 30% (B) at 15 min; and 90% eluent (A) and 10% (B) at 16–18 min. The chromatograms were obtained at 237, 257, 282, and 358 nm. The rutin present in the samples was identified based on the retention time and the UV-Vis spectrum in comparison with the spectra obtained for rutin standards and following the literature. The data were collected and treated using Chromeleon software, version 7.2 SR4.

2.5. Preparation of Oil-in-Water Semisolid Formulation

2.5.1. Preparation of Base Formulations

There were 3 semisolid formulations developed in order to choose the best one for the extract incorporation. The composition of each formulation is detailed in Table 1.

Table 1. Qualitative and quantitative composition of the 3 base formulations.

Components	Function	Quantity (g)		
		Formulation 1	Formulation 2	Formulation 3
Hydrophobic phase				
Lanette N®	Emulsifying agent O/A	10	12	14
Cetiol V®	Hydrophobic excipient and emollient	15	17	19
White petrolatum	Hydrophobic excipient and emollient	5	6	7
Hydrophilic phase				
Glycerin	Hydrophilic excipient; moisturizing and emollient agent	10.50	10.50	10.50
Water	Aqueous solvent	q.s. ¹ 100 g	q.s. ¹ 100 g	q.s. ¹ 100 g
Methylparaben	Preservative	0.1	0.1	0.1

¹ q.s.—quantum satis.

The base formulations were classified as O/W creams consisting of 2 phases, a hydrophilic and a hydrophobic phase. The hydrophilic phase contained water, glycerin, and methylparaben, while the hydrophobic phase was composed of Lanette N®, Cetiol V®, and white petrolatum.

For the cream preparation, the hydrophobic phase components, namely Lanette N®, Cetiol V®, and white petrolatum, were placed in a porcelain capsule and melted in a water bath at 60–80 °C. Afterwards, in a glass beaker, the methylparaben was dissolved in the glycerin, and water was added. The mixture was heated in a water bath at 60–80 °C.

When both phases were at the same temperature, the hydrophobic phase was added to the hydrophilic phase and the mixture was mixed thoroughly using a glass rod. After turning off the heating, the formulations were slowly homogenized at room temperature until cooling, then packed in glass jars (batches of 100 g), and stored at 25 ± 1 °C.

2.5.2. Preparation of a Formulation Containing *C. sativa* Shells Extract

The base formulation used to prepare the O/W cream containing *C. sativa* shells extract was selected based on the best outcomes obtained for the physical and technological properties. Table 2 summarizes the composition of the formulation incorporating *C. sativa* shells extract.

Table 2. Composition of the O/W formulation containing *C. sativa* shell extract.

Components	Quantity (g)
<i>Hydrophobic phase</i>	
Lanette N [®]	12.00
Cetiol V [®]	17.00
White petrolatum	5.00
<i>Hydrophilic phase</i>	
Glycerin	10.50
<i>C. sativa</i> shells extract	55.40
Methylparaben	0.10

The O/W cream incorporating *C. sativa* shells extract was prepared following the same procedure described for the base formulations. The chestnut shells aqueous extract replaced the water added in the base formulations. The formulations were packed in glass jars, in batches of 100 g, and rested for 24 h before any measurement. There were 3 batches of formulation prepared and stored at 25 ± 1 °C.

2.6. Characterization of the Oil-in-Water Semisolid Formulation Containing *C. sativa* Shells Extract

2.6.1. Color Evaluation

The color was evaluated using a colorimeter (Chroma Meter CR-410, Konica Minolta, Tokyo, Japan), previously calibrated with a white reference background, with a D₆₅ light source and an observation angle of 2°. An aliquot of the formulation was placed on a Petri plate with a 5.5 cm diameter and a sample thickness of 5 mm. The results were expressed according to the color space CIE 1976 L* a* b*, defined by the *Commission Internationale de l'Éclairage* (CIE). The 3 coordinates of color represent the lightness (L*), the chromatic scale between red and green (a*), and the chromatic scale between yellow and blue (b*). The data were collected using the Spectra MagicTM NC software. All measurements were made in triplicate.

2.6.2. Determination of pH

The pH was determined using a pH meter (Basic 20+, Crison Instruments, Barcelona, Spain) equipped with a glass electrode previously calibrated with 3 buffer solutions, acidic (pH 4.0), neutral (pH 7.0), and basic solutions (pH 9.2). First, an aliquot of formulation (1 g) was diluted in 3 mL of distilled water. The pH of the obtained solution was then determined. The measurements were performed in triplicate for each sample.

2.6.3. Determination of Droplet Size

The droplet size of the internal phase of the O/W formulations was evaluated by laser diffraction using a Mastersizer 3000 Malvern[®] device assembled to an automated sample's dispersion unit Hydro EV (Malvern[®], UK). The experimental conditions were defined as follows: particle refractive index of 1.2, particle absorption index of 0.01, and refractive

index of dispersant of 1.33. Water was used as a dispersant. Mie's model was employed as a light scattering model. Briefly, the sample was added into a glass beaker containing water until reaching an obscuration interval varying from 5 to 10%. For each sample, the equipment made 5 consecutive readings of the droplet size and expressed the final results as the mean and standard deviation. The results were represented in a graph of density in volume (%) as a function of the droplet size (μm) and expressed in 3 values, Dv10, Dv50, and Dv90, corresponding to 10th, 50th, and 90th percentiles, respectively.

2.6.4. Rheological Behavior Analysis

The rheological properties were assessed on a rotational rheometer (Kinexus lab+, Malvern, Worcestershire, UK) with a plate–plate configuration (geometry PU20 SR4367) with a 1 mm gap at 25 °C (Peltier plate cartridge). The samples were placed in appropriate amounts to entirely fill the space between the dishes. This equipment allows for the study of the flow and deformation of matter, measuring the viscosity and elasticity of non-Newtonian fluids under a wide range of conditions, and establishing an interrelation between deformation, force, and time. The assessment of rheological properties of semisolid formulations is of uttermost importance in the evaluation of their quality and stability. The data were collected using the rSpace software (Kinexus 1,75: PSS0211-17).

Viscosity

The viscosity of a formulation reflects its resistance to flow. For instance, the higher the viscosity, the lower the flow speed of the formulation [16]. The viscosity analysis was performed at 25 ± 1 °C in a shear rate range from 0.1 to 100 s^{-1} , with 10 samples per decade. The final results were represented in a graph that relates the apparent viscosity (mPa.s) with the shear rate (s^{-1}).

Thixotropy

Under constant shear stress, fluids may exhibit changes in viscosity as a function of time, being classified as thixotropic or anti-thixotropic. The thixotropy refers to the capacity of a formulation to recover its initial structure after ceasing the shear stress applied [17,18]. A formulation with thixotropic behavior tends to be physically more stable since its constituents are hardly separated [19]. The thixotropy was evaluated in 3 stages; the first stage occurred at a shear rate of 0.1 s^{-1} for 60 s, the second stage at 100 s^{-1} for 30 s, and the third stage at 0.1 s^{-1} for 600 s.

Yield Stress

The yield stress is the minimum stress required to initiate the flow. For instance, above the yield stress value, the matter flows easily [16,18,19]. The yield stress has great relevance when a formulation is intended to maintain its shape until reaching enough tension to be spread on the skin [19]. The yield stress was determined at time 0 under a shear stress that varied from 0 Pa to 20 Pa, for 1 min and 40 s.

2.7. Stability Study

The formulation containing the extract was evaluated at different storage times, namely at time 0 (T0) and after 15 and 30 days (T15 and T30, respectively), stored at controlled temperature (25 ± 1 °C). The parameters analyzed were color, pH, droplet size, and viscosity.

2.8. Statistical Analysis

All experimental analyses were carried out at least in triplicate. The results were reported as the mean \pm standard deviation of at least triplicate experiments. IBM SPSS Statistics 24.0 software (SPSS Inc., Chicago, IL, USA) was employed to perform the statistical analysis of the data. One-way ANOVA was applied to investigate the differences between samples for all assays and *post hoc* comparisons of the means were performed

with Tukey's HSD test. A denoting significance was accepted for $p < 0.05$ in all cases. In Tables 3–5, different letters (a, b, c) in the same column indicate significant differences between the results.

3. Results and Discussion

3.1. Quantification of Rutin in *C. sativa* Shells Extract by HPLC-UV-Vis

Chestnut shells have been described as a biowaste rich in phenolic compounds that are endowed with interesting pro-healthy properties that have captured the attention of food researchers and industry [8,12,13,19]. De Vasconcelos et al. reported high amounts of phenolic compounds in 70% methanol extracts obtained from chestnut shells of different cultivars (58.41–126.75 mg/g on fresh weight) [8]. Hydrolyzable tannins were the major phenolic class, namely vescalagin, castalagin, and acutissimin, followed by phenolic acids (mostly gallic and ellagic acids) [8]. In another study, Aires et al. also attested the richness of chestnut shells aqueous extracts in phenolic compounds (433.1–578.8 µg/g of dry weight (dw)), including gallic acid, ellagic acid, catechin, epicatechin, and epigallocatechin [13]. Squillaci et al. reported considerable levels of two flavonoids, catechin (0.70 mg/g dw) and epicatechin (0.71 mg/g dw), in aqueous extracts from chestnut shells [12]. Rutin was first identified by Nazzaro et al. as one of the main phenolic compounds that contribute to the bioactivity of chestnut shells [20]. In addition, Almeida et al. described rutin as the most active phenolic compound in chestnut leaf hydroalcoholic extract and incorporated it into an antioxidant topical formulation [19]. The authors also proved the in vitro release of rutin from the formulation for 6 h using Franz diffusion cells [19]. Moreover, UAE has been proposed as a sustainable option to valorize food by-products, such as chestnut shells, as promising substrates for different industries, including cosmetics. In our previous study, we characterized the phenolic composition of UAE extract obtained from chestnut shells [15]. Besides ellagic acid (40.4 µg/g dw), flavonoids were also quantified in substantial amounts, including epigallocatechin (15.3 µg/mg dw), catechin/epicatechin (7.4 µg/mg dw), apigenin-7-O-rutinoside (0.8 µg/mg dw), and luteolin-7-O-rutinoside (1.1 µg/mg dw) [15].

Rutin is a common dietary flavonoid, namely classified as a flavonol glycoside, abundantly present in fruits, vegetables, and plant-derived products. This phenolic compound provides interesting pro-healthy effects in preventing diseases and protecting genome stability [21]. Different studies have demonstrated the pharmacological activities of rutin, including anticancer, antidiabetic, antioxidant, anti-inflammatory, antimicrobial, and neuro-protective properties, as beneficial for the treatment of several pathological conditions, such as varicose veins, internal bleeding, or hemorrhoids [21–23]. For instance, Choi et al. attested the anti-aging effects of rutin in dermal fibroblasts and human skin [24]. The authors suggested that rutin upregulates mRNA expression of collagen type I and downregulates mRNA expression of matrix metalloproteinase 1 (MMP1), as well as stimulating ROS scavenging in fibroblasts. An in vivo clinical study in 40 subjects (30–50 years) applying a rutin-containing cream for 4 weeks showed an increase of skin elasticity and reduction of number, length, and areas of wrinkles [24]. For this reason, numerous commercial formulations have incorporated rutin in different dosages as active ingredients [21]. Based on that, in this study, rutin was identified and quantified in *C. sativa* shells extract by HPLC coupled with a UV-Vis detector. Prior to quantification, the maximum and minimum absorptions for the standard rutin solution were determined (Figure 1).

Rutin was identified in *C. sativa* shells extract at 282 nm, based on retention time (7.27 min) and the UV-Vis spectrum by comparison with rutin standards following the methodology validated by Almeida et al. [25].

For the quantification of rutin, a calibration curve was plotted using rutin standards at a concentration range of 1.36–10.08 µg/mL. The coefficient of determination (R^2) was 0.9983 calculated from Equation (1) of the rutin calibration curve:

$$Area = 0.1227 \times [Rutin] + 0.0095 \quad (1)$$

The amount of rutin quantified in *C. sativa* shells extract was 1.50 ± 0.07 mg/g of plant material on dry weight (dw). Nazzaro et al. also investigated the phenolic composition of *C. sativa* shells extracts obtained by conventional extraction (at room temperature for 5 days using solvents with different polarities) [20]. Lower levels of rutin (0.05 mg/g dw) were determined in methanol/water (70:30) extract when compared to the extract prepared in this study. Otherwise, rutin was not identified in aqueous and methanolic extracts [20]. The employment of UAE as a green and more efficient technique as well as the application of higher temperatures may explain the higher recovery of rutin in the extract prepared in the present study.

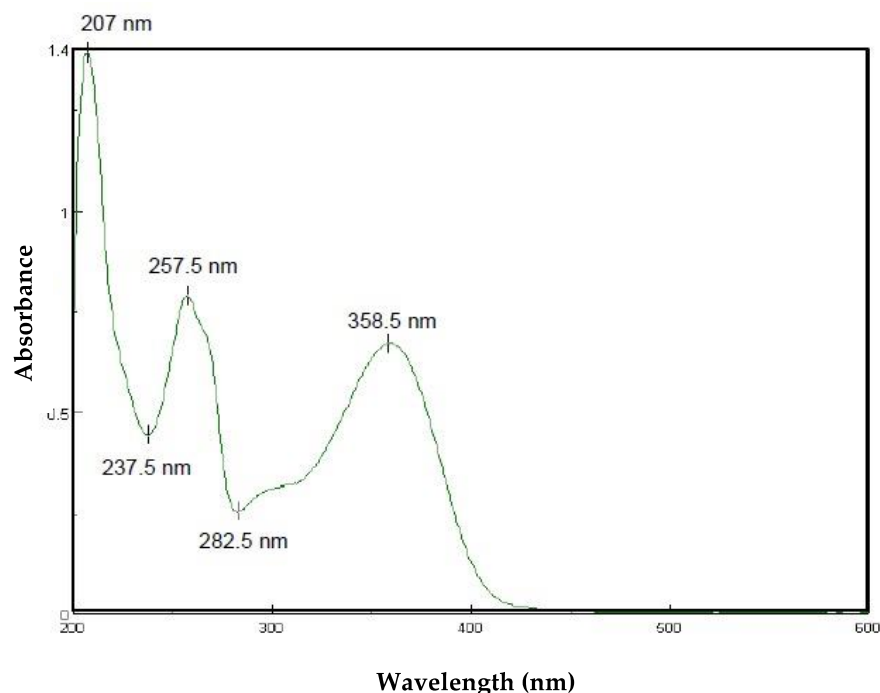


Figure 1. Absorption spectrum (absorbance vs. wavelength) of rutin obtained at the wavelength range of 200–600 nm (UV–Vis region).

Likewise, Almeida et al. reported rutin as the major phenolic compound (5.9 mg/g dw) identified in ethanol/water (7:3) extract from chestnut leaves, this result being higher than the one obtained in this study [25]. These differences are probably due to the higher exposure of leaves to climatic changes when compared to shells. Indeed, phenolic compounds, particularly flavonoids, are produced by plants in response to biotic and abiotic stress, such as UV radiation, light, temperature, water availability, and pathogen attacks, and are recognized as defense compounds of uttermost importance [26]. Shells are located inside burs and are less exposed to these abiotic factors.

In another study, Yang et al. identified and quantified rutin in *Euonymus alatus* stem extracts obtained by conventional extraction and UAE [27]. The results demonstrated that UAE was a more proficient technique based on the higher rutin levels (0.299 mg/g dw) when compared to maceration (0.157 mg/g dw). Another positive aspect was that the extraction time was 4 times shorter in UAE compared to conventional extraction. A possible explanation for these results may be related to the fact that rutin extraction by heating, boiling, or reflux can often lead to its degradation due to ionization, hydrolysis, and oxidation during extraction [27].

Considering the stability and quality of the semisolid formulation incorporating chestnut shells extract, further studies will assess the rutin content in the cream over time (at least 6 months) to appraise if any changes occur in the bioactivity of the final product during the storage. The quantification of rutin content in the cream through a longer

storage time might also be a relevant analysis to investigate its stability profile. Overall, an exhaustive assessment of the antioxidant properties and the main active compounds in the formulation that may contribute to its bioactivity should be provided in order to prove the claims as an antioxidant topical formulation.

3.2. Selection of the Best Base Formulation

The first step for the incorporation of chestnut shells extract in cosmetic products was the development of three base formulations with different compositions and the selection of the best one considering the physical and technological features. All three formulations presented a homogenous appearance without phase separation, a white color, and the absence of odor. Concerning the organoleptic properties, Formulation 2 showed the most suitable consistency, smoothness after skin application, and low oiliness, and was selected as the best base formulation to later incorporate the chestnut shells extract. In contrast, Formulation 3 was thicker, which hinders its ability to spread on the skin, while Formulation 1 had an exceedingly liquid consistency. These organoleptic properties remained unchanged after 15 and 30 days of storage. Furthermore, other technological properties were evaluated for the three formulations, namely pH, droplet size, and viscosity (Table 3; Figure 2).

Table 3. Droplet size (Dv10, Dv50, and Dv90) and pH variation of the 3 base formulations (mean \pm standard deviation, $n \geq 3$).

Base Formulations	Droplet Size			pH
	Dv10 (μm)	Dv50 (μm)	Dv90 (μm)	
1	13.2 \pm 0.35 ^a	39.7 \pm 0.47 ^a	79.6 \pm 1.53 ^a	5.44 \pm 0.16 ^a
2	13.3 \pm 0.35 ^a	40.0 \pm 0.57 ^a	89.7 \pm 1.74 ^a	5.47 \pm 0.05 ^a
3	11.9 \pm 0.28 ^b	36.4 \pm 0.84 ^b	143.0 \pm 13.0 ^b	5.46 \pm 0.13 ^a

Different letters (a, b, c) in the same column indicate significant differences ($p < 0.05$) between base formulations.

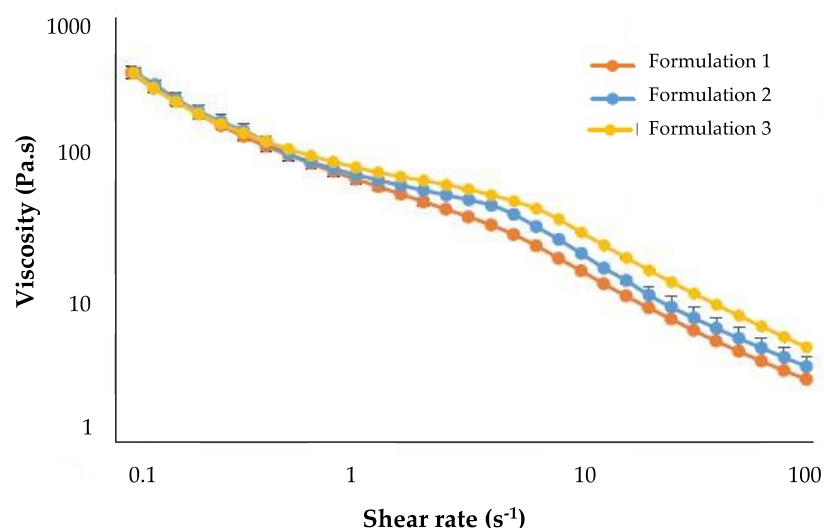


Figure 2. Graphic that represents the variation of shear viscosity (Pa.s) with shear rate (s^{-1}) for the 3 base formulations (mean \pm standard deviation, $n = 3$).

No significant differences ($p > 0.05$) on pH were observed between the three base formulations, presenting all formulations with a pH compatible with skin (pH = 4–6). Regarding the droplet size, the three formulations evidenced the presence of 90% of droplets with micrometric scale sizes. The statistical analysis demonstrated no significant differences ($p > 0.05$) in droplet diameters of Base Formulations 1 and 2. Nevertheless, the droplet sizes of Base Formulation 3 were statistically different ($p < 0.05$) from Formulations

1 and 2. Moreover, the viscosity profile of the three base formulations pointed out the non-Newtonian character, particularly a pseudoplastic behavior. Base Formulation 1 showed the lowest viscosity for shear rates above 0.900 s^{-1} , while Base Formulation 3 displayed the highest viscosity among the three formulations, which explains the greater difficulty in skin application. The higher viscosity of Formulation 3 may be due to the presence of higher amounts of Lanette N[®], Cetiol V[®], and white petrolatum. Considering all parameters, Base Formulation 2 was selected to incorporate the extract as an active ingredient with the aim to produce an antioxidant-enriched semisolid formulation intended for cosmetic purposes.

3.3. Technological Characterization of Semisolid Formulation Containing *C. sativa* Shells Extract

The semisolid formulation with the extract exhibited a brownish pink color, a fruity odor, and a homogeneous appearance. The stability of the organoleptic features was also attested, based on the similar organoleptic properties observed at time 0 and after 15 and 30 days of storage.

3.3.1. Color Evaluation

The color was evaluated by instrumental analysis to appraise the consumer acceptance of the cosmetic product along with the sensorial assessment [28]. Table 4 presents the results of color (for each coordinate of the color system CIE L*a*b*) of the semisolid formulation with chestnut shells extract.

Table 4. Color (L*, a* and b*) and pH variation of semisolid formulation containing *C. sativa* shells extract evaluated at different storage times (mean \pm standard deviation, $n = 3$).

Formulation with <i>C. sativa</i> Shells Extract	Color			pH
	L* (Lightness)	a* (Redness)	b* (Yellowness)	
T0	77.96 \pm 0.34 ^a	5.07 \pm 0.09 ^a	16.63 \pm 0.20 ^a	5.28 \pm 0.04 ^a
T15	75.55 \pm 0.01 ^b	6.33 \pm 0.01 ^b	18.93 \pm 0.01 ^b	5.29 \pm 0.04 ^a
T30	74.34 \pm 0.01 ^c	7.26 \pm 0.02 ^c	21.40 \pm 0.01 ^c	5.36 \pm 0.03 ^a

Different letters (a, b, c) in the same column indicate significant differences ($p < 0.05$) between the different times.

The color of the O/W cream with chestnut shells extract was compared to the Base Formulation 2. Considering the L* parameter, both formulations showed high lightness with values ranging between 77.96 and 90.27 for the formulation with extract and base cream, respectively. However, a significant decrease ($p < 0.05$) of L* was detected after adding the extract to the base formulation. Regarding a* and b*, the formulation with extract exhibited positive values associated with the prevalence of redness and yellowness. Oppositely, Base Formulation 2 showed negative results for a* (−0.61) and b* (−1.07), suggesting the prevalence of greenness and blueness. Significant differences ($p < 0.05$) were observed between the base and extract-loaded formulations for all color coordinates. Considering that consumers prefer cosmetics with warm colors and mild odors, the O/W cream containing the extract displayed favorable organoleptic properties that may contribute to a good consumer acceptance.

The stability study of the formulation with extract over 30 days of storage also showed significant changes ($p < 0.05$) on the three-color coordinates, suggesting a major influence of time on color. A significant reduction of L* indicated that the formulation had a lower lightness after 30 days of storage. Concerning a* and b*, the values increased significantly with storage time, but the prevalence of redness and yellowness remained.

Recently, Pinto et al. prepared hydrogels containing different percentages (25–89%) of *C. sativa* bur hydro-alcoholic extract [28]. The hydrogels exhibited lower lightness (10.01–12.44) as well as a lower prevalence of redness (−0.73–2.74) and yellowness (1.69–6.71) compared to the cream formulated in the present study. Over 30 days of storage, L* and b* varied significantly ($p < 0.05$), while a* showed similar results for all formulations at different storage conditions [28]. Almeida et al. also prepared a hydrogel containing *C. sativa* leaf extract as an active ingredient, describing a lower lightness (around 65), similar results for

b* (around 20), and negative results for a* (around −4), which indicated the predominance of greenness [19]. The authors reported similar results for L* and b* over 1 month of storage, while slightly lower a* values were obtained after 1 month when compared to the initial measurements [19]. In another study, Censi et al. developed O/W emulsions containing açai extract (0.5–2%) and reported lower L* (66.98–58.46) and b* values (1.75–4.51) [29]. However, similar a* outcomes were obtained for preparations containing between 0.5 and 1% of açai extract (5.49–11.30) [29].

3.3.2. Determination of pH

The pH of O/W cream containing *C. sativa* shells extract was determined at different storage times (T0, T15, and T30) (Table 4).

Compared to the Base Formulation 2 (pH = 5.47), the cream with extract showed a slightly lower pH (5.28) which may be explained by the incorporation of the extract that displayed a more acidic pH (4.36). Significant differences ($p < 0.05$) were observed between the pH of the extract, the base formulation, and the formulation with extract. Considering that skin pH ranges between 4 and 6, the O/W cream with chestnut shells extract was revealed to be compatible with skin. Overall, the results highlighted the potential of this formulation to be marketed by the skin care industry.

Concerning the storage time, similar pH values were determined for extract formulation at time 0 and after 15 and 30 days without significant differences ($p > 0.05$). In regard to the pH, the stability of the O/W formulation with extract was ensured for 30 days at 25 °C, and was the formulation considered suitable for cutaneous application.

Likewise, Pinto et al. reported a similar pH (5.67) for the hydrogel incorporating 50% of chestnut bur extract [28]. However, significant pH changes ($p < 0.05$) were detected after 30 days of storage at 20 °C [28]. Opposite to the present study, a hydrogel with *C. sativa* leaf extract presented a substantially lower pH (4.73–4.76), but this formulation was still compatible with skin [19]. In addition, a slightly lower pH (5.02–5.05) was obtained for emulsions with açai extract (0.5–2%) [29]. Athikomkulchai et al. formulated a cream containing *Moringa oleifera* seed oil as an active agent, describing a pH of 5.43 in comparison with the base cream (5.07) [30].

3.3.3. Determination of Droplet Size

Table 5 presents the droplet diameters of Base Formulation 2 and the O/W cream with chestnut shells extract, determined by laser diffraction.

Table 5. Evaluation of droplet sizes of Base Formulation 2 and O/W cream containing *C. sativa* shells extract at time 0 and after 30 days of storage (mean \pm standard deviation, $n = 5$).

	Storage Time (Days)	Droplet Size		
		Dv10 (μm)	Dv50 (μm)	Dv90 (μm)
Base Formulation 2	T0	13.30 \pm 0.35 ^b	40.00 \pm 0.57 ^b	89.70 \pm 1.74 ^c
Formulation with <i>C. sativa</i> shells extract	T0	12.20 \pm 0.41 ^c	35.00 \pm 0.57 ^c	130.00 \pm 8.77 ^a
	T30	15.50 \pm 0.30 ^a	50.50 \pm 1.00 ^a	111.00 \pm 6.43 ^b

Different letters (a, b, c) in the same column indicate significant differences ($p < 0.05$) between the different times.

The droplet sizes of the formulation containing chestnut shells extract and the Base Formulation 2 were compared in order to estimate possible changes after incorporating the extract. In addition, the stability of the formulation with the extract regarding the droplet size was appraised at time 0 and after 30 days of storage (25 \pm 1 °C).

Considering the results for the formulation containing extract, the median size (Dv50) of the droplet population was 35 μm (at time 0). In addition, 90% of the droplets (Dv90) presented a size not bigger than 130 μm , while 10% of the droplets (Dv10) were no more than 12.20 μm in diameter. In regard to Base Formulation 2, a slightly higher median size was obtained, with 50% of the droplets presenting bigger than 40 μm in diameter. Only

10% of the droplets showed a size bigger than 89.70 μm , taking into consideration the Dv90 value, whereas another 10% of the droplets (Dv10) did not exceed 13.30 μm in diameter. The statistical analysis revealed significant changes ($p < 0.05$) in droplet size between Base Formulation 2 and the O/W cream with extract.

The stability study of the formulation containing the *C. sativa* shells extract demonstrated a significant increase ($p < 0.05$) of droplet size over 30 days of storage, aligning with the Dv10 and Dv50 results. However, the Dv90 value indicated a significant reduction ($p < 0.05$) of the droplet size over storage time. Besides the considerable changes in droplet size, the physical and technological stability of the formulation was not compromised since the intended application is for skin and cosmetics' droplets do not need to have small sizes once they are concentrated mainly in the upper skin layers [19].

3.3.4. Rheological Studies

Viscosity

When a new formulation intended for cutaneous application is developed, the viscosity should be assessed, not only to appraise the consumer acceptance but also as a mandatory parameter to estimate its stability [19,31]. Viscosity can be defined as the resistance of a formulation to deformation or flow, varying with the shear stress and relying on physicochemical properties and temperature [16,18]. Considering the rheological behavior, formulations may be classified as Newtonians or non-Newtonians [16,18]. A formulation with a Newtonian profile presents a constant viscosity independent of the shear rate, whereas non-Newtonian systems show a variation of viscosity with the shear rate, and the viscosity is also referred to as the “apparent viscosity” [16,18]. Among non-Newtonian systems, a formulation may have a pseudoplastic (shear thinning) or dilatant behavior (shear thickening) [19]. Regarding the pseudoplastic systems, a reduction of apparent viscosity is observed for high shear rates, whilst an augment of viscosity at high shear rates characterizes the dilatant formulations [16,18]. Figure 3 represents the rheogram that relates the shear viscosity (Pa.s) with the shear rate (s^{-1}) of the semisolid formulation containing *C. sativa* shells extract at different storage times.

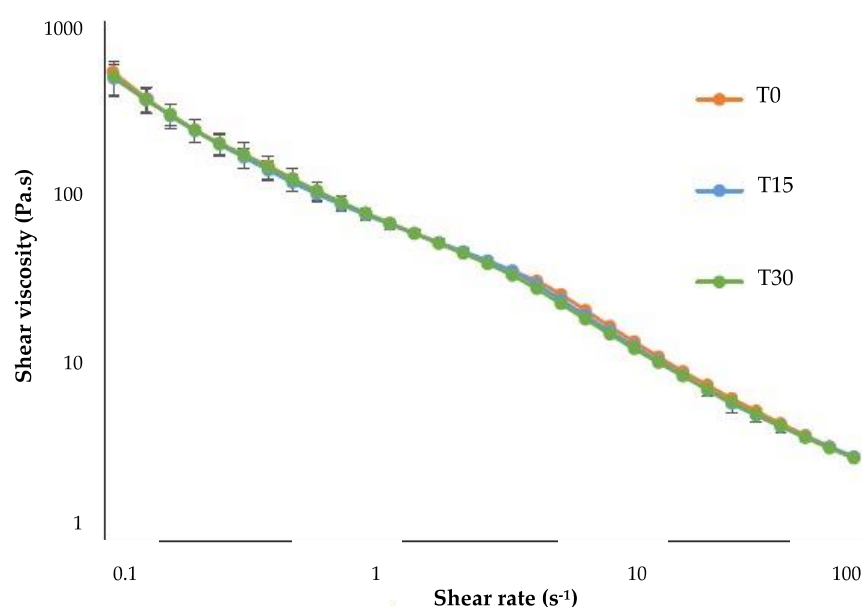


Figure 3. Graphic that represents the variation of shear viscosity (Pa.s) with shear rate (s^{-1}) for O/W formulation containing *C. sativa* shells extract at different storage times (T0, T15, and T30) (mean \pm standard deviation, $n = 3$).

As shown in Figure 4, a decrease in the apparent viscosity was observed with the increase in the shear rate, suggesting that the formulation with chestnut shells extract has a

non-Newtonian behavior, namely pseudoplastic. The pseudoplastic behavior of cosmetic formulations is favorable for skin application since the force required to allow the spreading of the formulation is relatively low and drops when it is applied to the skin [18,19]. This rheological behavior is characteristic of water-in-oil emulsions and gels prepared with acrylic polymers [19,32]. Previous studies described a similar rheological profile for hydrogels containing *C. sativa* leaf and bur extracts [19,28]. Likewise, Athikomkulchai et al. related a pseudoplastic character for a cream incorporating moringa seed oil [30]. In addition, no significant changes ($p > 0.05$) were detected in the viscosity of creams containing açai extract [29].

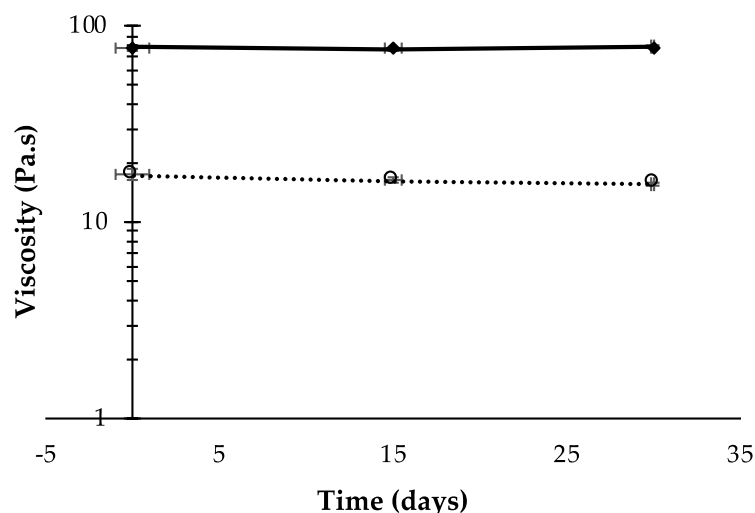


Figure 4. Graphic representing the variation of viscosity (Pa.s) vs. time of the formulation with *C. sativa* shells extract at a shear rate of 1.070 s^{-1} (upper line) and a shear rate of 10.060 s^{-1} (lower line) (mean \pm standard deviation, $n = 3$).

Concerning the stability over storage time, the viscosity of the O/W cream with extract maintained unchanged after 15 and 30 days, evidencing similar rheological character. In this sense, time did not seem to significantly influence ($p > 0.05$) the viscosity. Figure 4 illustrates the variation of viscosity (at shear rates of 1.070 s^{-1} and 10.060 s^{-1}) of the semisolid formulation with extract through 30 days of storage.

Furthermore, the high viscosity of a semisolid formulation may exert a positive effect on the extract stability by decreasing the diffusion rate of oxygen [19]. The unchanged viscosity over storage time ($p > 0.05$) and similar rheological behavior indicated that the formulation with chestnut shells extract was stable regarding viscosity for 30 days at 25°C .

Analysis of Thixotropy

Thixotropy is described as a time-dependent shear-thinning property and is characterized by a reversible structural transition caused by alterations on apparent viscosity [17]. Two stages define the thixotropy: fluidification (occurs at high shear rates) and stiffening (happens in rest or at low shear rates) [17]. A formulation with thixotropic behavior exhibits a reduction of apparent viscosity (or the corresponding shear stress) over time, with its microstructure responding well to the flow, becoming more fluid after applying a force and breaking down by deformation [17,19]. Oppositely, the formulation recovers its initial viscosity when returning to the rest state. Concerning formulations with anti-thixotropic characteristics, an increase of the apparent viscosity occurs when force is applied [17,19]. Beyond the non-Newtonian behavior, most cosmetic formulations also possess thixotropic character [19]. Figure 5 represents the relationship between shear viscosity (Pa.s) and time of deformation (s) for the O/W cream containing chestnut shells extract.

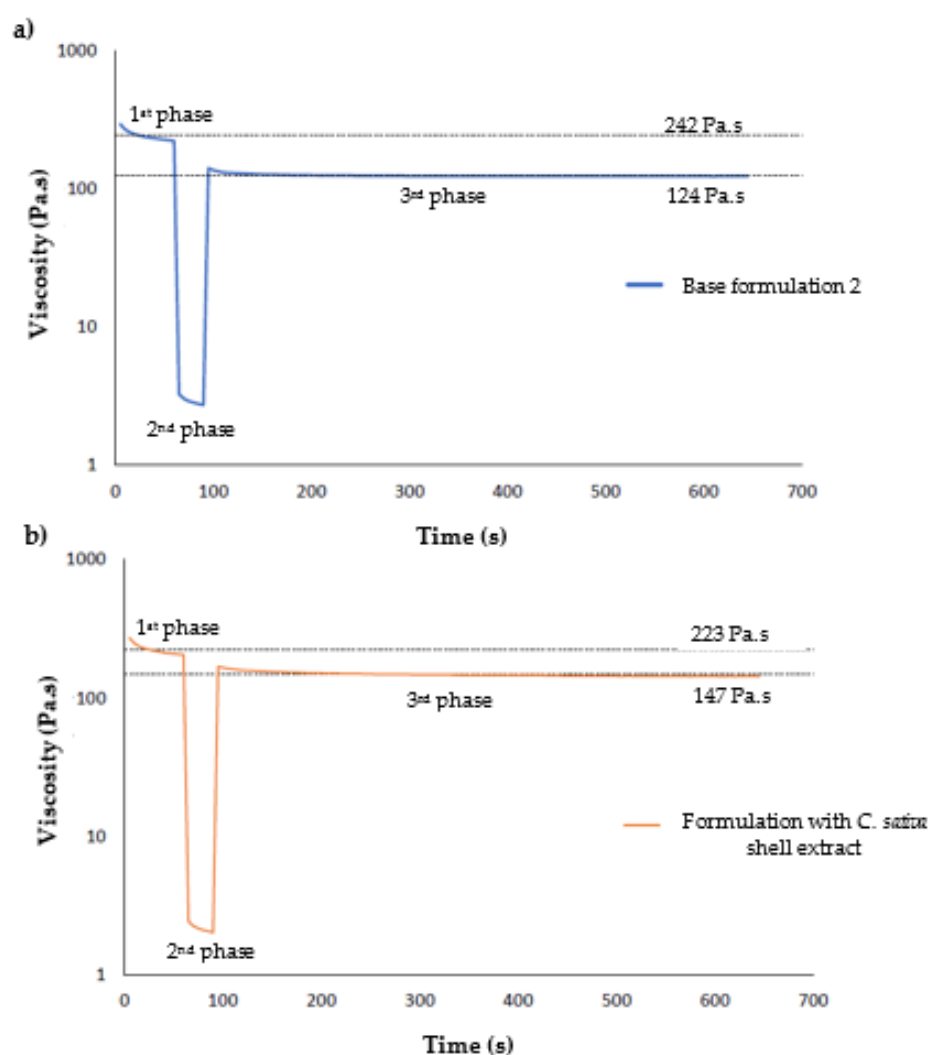


Figure 5. Graphic that represents the variation of the viscosity (Pa.s) with time of deformation (s) for Base Formulation 2 (a) and O/W formulation containing *C. sativa* shells extract (b), at a shear rate from 0.1 to 100 s^{−1} and returning to 0.1 s^{−1} (mean ± standard deviation, *n* = 3).

As depicted in Figure 5, the base formulation and the O/W formulation containing chestnut shells extract evidenced thixotropic behavior. Three distinctive phases were identified. In the first phase, the formulations were slightly disturbed by the rotational movements of one of the two parallel plates at a shear rate of 0.1 s^{−1}, and the viscosity remained almost constant. In the second phase, an abrupt reduction of viscosity was observed with the increase in shear rate to 100 s^{−1} and, consequently, the formulations became more fluid. Low viscosity under medium to high shear rates is favorable for an easy application of the formulation on skin. Finally, in the third phase, the formulations partially recovered the initial viscosity after the shear rate decreased back to 0.1 s^{−1}. The recovery rate of the initial viscosity was 51.2% and 65.9%, respectively, for the Base Formulation 2 and the O/W cream containing chestnut shells extract. The high viscosity at rest prevents undesirable effects such as phase separation and, accordingly, extends the shelf life of the product. In addition, a delayed recovery of the initial viscosity enhances the absorption of formulation through the skin. The thixotropic behavior of cosmetic formulations allows for easier spreading of the product on skin.

For instance, Censi et al. also described a thixotropic profile for O/W emulsions incorporating açai extract, emphasizing the marked viscosity at higher shear rates and no total recovery of the initial viscosity after ceasing the shear rate [29]. Athikomkulchai

et al. underlined the shear-thinning behavior of *M. oleifera* seed oil cream without a yield stress, suggesting that the increase of shear rate when the formulation is applied may lead to a low resistance to flow and the release of moringa seed oil to the skin [30]. In contrast, Almeida et al. reported that the hydrogel containing chestnut leaf extract did not exhibit pronounced thixotropy [19].

Yield Stress

The yield stress is the minimum tension required to prompt a plastic deformation without a considerable augment in load [16]. The yield stress of the base formulation and the cream with *C. sativa* shells extract were, respectively, 24.66 and 37.04 Pa. The incorporation of extract into the base formulation led to a significant increase ($p < 0.05$) of the minimum shear stress required to induce the flow of the formulation. Overall, the results indicate that the semisolid formulation with extract needs a higher tension to drain.

On the other hand, Almeida et al. reported a higher yield stress (101.34 Pa at time 0) for a hydrogel containing chestnut leaf hydroalcoholic extract, meaning that this formulation needed even greater tension to flow [19]. However, the authors described a considerable decrease of yield stress after 6 months of storage at 20 °C (57.01 Pa).

3.4. Stability Study during Storage Time

The stability study during storage time was performed at time 0 and after 15 and 30 days of storage at 25 °C for organoleptic properties, color, pH, droplet size, and viscosity. The formulation containing chestnut shells extract exhibited similar organoleptic properties, pH, and rheological behavior at time 0 and after 15 and 30 days of storage without significant changes ($p > 0.05$), emphasizing the stability of the O/W cream with extract over 1 month. Oppositely, significant changes ($p < 0.05$) to droplet size and color parameters (L^* , a^* , and b^*) were observed throughout 30 days of storage.

Although minor changes were observed regarding color and droplet size over the storage time, taking all the outcomes together, it is possible to conclude that the formulation containing the extract exhibited a good physical and technological stability when stored for 30 days at 25 °C. In general, the stability of the formulation was not compromised by the small differences in color and droplet size, but these parameter changes may have an impact on consumer acceptance.

The incorporation of antioxidants extracted from natural sources, particularly food by-products, in cosmetic formulations represents a huge challenge due to the high instability of these ingredients in aqueous systems [31]. The physical stability may be influenced by the chemical instability of some added constituents. Indeed, numerous factors may lead to phenolic compounds' degradation, such as the hydrophilic phase of formulation, the oxygen, the storage aging, and the temperature, which may affect the stability of the semisolid formulations [31,33]. Further studies should evaluate the impact of longer storage, namely 3 and 6 months, on the physical, functional, and microbiological properties of the formulation. Regarding the functionality of phytochemicals from *C. sativa* shells, possible interferences with other constituents of the formulation should be also investigated. In addition, in vivo efficacy and sensorial studies of this new formulation must be demonstrated prior to its use as a cosmetic.

As stated by the European Regulation No. 1223/2009, the safety and stability of formulations is a top priority in the approval of the commercialization of cosmetic products. In this sense, the effectiveness of the semisolid formulation developed as an antioxidant topical cream containing chestnut shells extract should be guaranteed through storage, evidencing the stability of the active ingredients, excipients, and, consequently, the final product. Additionally, changes in color, pH, and viscosity may indicate the degradation of active substances and, accordingly, affect the effectiveness and stability of semisolid formulations [25,31,33]. For this reason, the evaluation of physicochemical characteristics not only complies with the European Cosmetic legislation but also provides an interesting marker of the stability and bioactivity of final products.

4. Conclusions

The current trend in the cosmetic industry encompasses the formulation of novel skin care products incorporating natural extracts as active ingredients. Natural antioxidants have been proposed not only as valuable alternatives to synthetic ones but also as anti-aging compounds with outstanding skin health-promoting properties, particularly in the prevention or treatment of oxidative stress-mediated dysfunctions (e.g., cancer, skin inflammatory diseases, and premature aging). The valorization of chestnut shells as a source of antioxidants could also have a huge impact, particularly considering the sustainability concerns due to the broad geographic distribution of this crop waste. The present study formulated an O/W cream incorporating chestnut shells extract rich in rutin, a quercetin glycoside endowed with antioxidant and anti-aging effects, whose efficacy in cosmetic creams was already demonstrated. The *C. sativa* shells cream developed in this work displayed favorable organoleptic properties that may contribute to good consumer acceptance. The stability of the formulation incorporating the chestnut shells extract was ensured for up to 30 days of storage, only evidencing minor changes in color and droplet size. Thus, the design of skin care formulations using *C. sativa* shells as a source of high added valued compounds comprises a new challenge for cosmetic companies, hypothesizing its beneficial properties for human skin. As far as we know, this is the first study that formulated a chestnut shells-containing cream as an alternative way to recycle this agro-residue. It is worth noting that in further studies, we will evaluate the stability of the semisolid formulation incorporating the chestnut shells extract at different times (for 3 to 6 months) and temperatures (2 °C and 45 °C).

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