Hemp-seed and olive oils: Their stability against oxidation and use in O/W emulsions

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Synopsis

Hemp-seed oil has several positive effects on the skin: thanks to its unsaturated fatty acid (PUFA) content it alleviates skin problems such as dryness and those related to the aging process. We present a comparative study of hemp-seed and olive oils, determining some physicochemical indices and evaluating their stability against oxidation. The peroxide value of hemp-seed oil was below 20, the threshold limit for edible oils. Hemp-seed oil was less stable against peroxidation than olive oil, but MDA and MONO assays showed its stability to be above expectations. The chlorophyll contained in extra virgin olive oil had a higher photostability than that contained in hemp-seed oil, possibly due to the larger amount of antioxidant in the olive oil. A certain amount of Vitamin E was found in hemp-seed oil. Since quality analyses indicated that hemp-seed oil is relatively stable, emulsions were prepared with the two oils, and their stability and rheological characteristics were tested. Some of the resulting gel–emulsions were suitable for spraying on the skin.

INTRODUCTION

Hemp (*Cannabis sativa* L.) is one of the oldest and most versatile plants (1–3). Documentation of its use dates back as far as the 28th century B.C., and it likely to have been used even earlier; the fiber was used for textiles and the seeds for food. The existence of its botanical relative, marijuana (cannabis varieties containing psychoactive substances) did not enhance the status of the plant. Since the late 1930s many Western countries have prohibited its cultivation due to the presence of the phytochemical drug component δ -9-tetrahydrocannabinol (THC). Then, in the late 1980s and early 1990s, hemp was rediscovered; today only varieties containing less than 0.3% THC in their flower portions can legally be farmed in Canada and the European Union, whereas marijuana flowers typically contain between 3% and 20%.

In addition to its nutritional value, hemp-seed has other health benefits, including lowering cholesterol and high blood pressure. Hemp seeds are a rich source of essential

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fatty and amino acids and contain other essential nutrients, such as the antioxidant vitamin E, minerals, and lecithin. Essential oil is one of the many products that may be obtained from hemp: it contains many volatile compounds, mainly monoterpenes, sesquiterpenes, and other terpenoid-like compounds that can be isolated by steam distillation. Several factors influence the yield and quality of hemp essential oil, including weather and harvesting techniques (4). The fatty-acid spectrum of hemp-seed oil is the cause of its nutritional value: compared to other unrefined edible oils, hemp-seed oil contains a very high percentage of essential fatty acids (EFA). More importantly, a high proportion (15% to 25%) is omega-3 alpha-linolenic acid, only found in significant quantities in a few other commonly used cooking oils, such as soybean and canola oils. In hemp-seed oil, the monounsaturated oleic acid contributes 10% to 15%, and total saturated fatty acids account for 9% to 11% of the total fatty acids. It also contains small percentages of several other polyunsaturated fatty acids (PUFAs) that play important roles in human metabolism; most notable are gamma-linolenic acid (GLA, 18:3 omega-6) and stearidonic acid (18:4 omega-3), whose content varies considerably with variety and growing conditions. The fat-soluble compounds of the vitamin E complex, tocopherols and tocotrienols, are other important constituents of hemp-seed oil; compared to other cold-pressed and unrefined vegetable oils-olive, sunflower, canola-it has a moderate to high content of vitamin E compounds. Typical levels are 100 to 150 milligrams per 100 grams of oil, predominantly gamma-tocopherol. This makes hempseed oil a valuable source of vitamin E, one to two tablespoons meeting daily requirements. Finally, unrefined hemp-seed oil also contains moderate amounts of several other beneficial or even essential constituents, such as phytosterols, phospholipids, carotenes, and several minerals including calcium, magnesium, and potassium.

Besides its nutritional benefits, hemp-seed oil has a number of positive effects on the skin: clinical studies have shown that PUFAs can alleviate or even resolve some skin problems, such as dry skin (5). Moreover, clinical trials with GLA supplementation have demonstrated a gradual improvement in the symptoms of a topical eczema and acne.

The rediscovery of hemp-seed oil has been accompanied by advertising campaigns for body-care products containing it, by the American and European companies that have developed and promoted related products since the early 1990s. Hemp-seed oil in body-care products protects the skin by reducing dryness and may slow the skin's aging process. Hemp is an eco-friendly crop requiring no biocides and little fertilizer in comparison with cotton, for which it is an excellent substitute. Industrial hemp products can also be substituted for products derived from fossil fuels, such as synthetic fibers (textile and rope), plastics and fuel, and wood-based products including paper and particle board (6).

Hemp-seed oil's high PUFA content and its high omega-3: omega-6 ratio are the main reasons for its health benefits; however, the same factors cause instability and mean that the product requires careful quality control throughout the production process since unsaturated fatty acids react with free radicals and oxygen to create numerous oxidation products. This gradual oxidation of fatty acids is called rancidification: oils turn rancid faster if exposed to oxygen, heat, and especially light. However, it has also been shown by differential scanning calorimetry (DSC) that hemp-seed oil has high kinetic stability during heating and cooling (7). Microwave treatment shifts the melting range of oils to lower temperatures and increases oxidation temperatures, suggesting an increased protective effect upon heating (7).

The physicochemical characteristics (8) of hemp-seed oil are examined through a number of analytical determinations: quality indices (free fatty-acid content, peroxide values, spectrophotometric characteristics in the UV region); parameters of oxidation processes (MDA and MONO analysis); and chlorophyll pigment content and its spectrophotometric characteristics in the UV region. Hemp-seed oil was compared with a commercial virgin olive oil, since olive oil is considered to be one of the most valuable edible fats, thanks to its nutritional characteristics. The rheological characteristics of different formulations of olive and hemp-seed oils were also investigated in order to determine the most suitable one for topical administration.

MATERIALS

Hamamelis virginiana distillate, cetearyl alcohol, hydrolyzed milk protein, hydroxy ethyl cellulose, Polysorbate 20 (Tween 20[®]), Polysorbate 40 (Tween 40[®]), Peg-40hydrogenated castor oil (Cremophor RH®40), and dimethicone (silicon oil 350 mPa s) were purchased from Acef. Acrylates/C10-30 alkyl acrylate crosspolymer (Carbopol 1382[®] and Pemulen TR-1[®]) were gifts from Biochim. C 14-22 alkylalcohol/C 12-20 alkylglucoside (Montanov L[®]), cetearyl alcohol/cetearyl glucoside (Montanov 68EC[®]), polyacrylamide, C_{13-14} isoparaffin, laureth 7 (Sepigel $305^{\text{(B)}}$), and Na lauroyl oat amino acids (Proteol OAT®) were gifts from Seppic. Methyl glucose sesquistearate (20) OE (Glucamate SS E20[®]), and methyl glucose dioleate (Glucate DO[®]) were gifts from Amerchol. Imydazolidynylurea (Gram I®), methylisothiazolinone/methylchloroisothiazolinone (Kathon CG[®]), magnesium chloride, and magnesium citrate were gifts from Sinerga S.r.l. Potassium hexadecyl hydrogen phosphate (Amphisol K®) was a gift from Roche. Poliglyceryl-3-methylglucose distearate (Tego Care 450[®]) was a gift from Goldschimdt Italia. Sucrose tristearate or palmitate (sucroesters DUB SE® 3S and 15P) and sucrose mono (or di)stearate (or palmitate) (sucroesters SP® 30 and 50) were gifts from Sisterna BV. C₁₂₋₁₅ alkyl benzoate (Finsolv TN®) was a gift from Prodotti Gianni. Glyceryl monostearate (Cutina GMS®) was a gift from Henkel. Olive leaf extract (Eurol BT®) and Olivem 700[®] were gifts from B&T S.r.l. Cannabis sativa seed oil was a gift from Verdesativa, and olive oil was purchased from Carli S.r.l. LRI® solubilizer was a gift from Waker. Acetic acid, hydrochloric acid, thiobarbituric acid, trichloracetic acid, butanol, ethanol, methanol, n-hexane, diethyl ether, phenolphthalein, potassium hydroxide, potassium iodide, sodium chloride, sodium hydroxide, and starch were from Merck. Chlorophyll 3A (1522), Chlorophyll (5022), Chlorophyll A10, and Chlorophyll B10 were from Biochim.

INSTRUMENTS

Viscosity of the oils was measured with a Schott-Geräte capillary viscometer (K = 0.01053, 0.4-6.0 cSt). An Abbie refractometer was used to characterize the oils, and a UV-VIS Lambda 2 spectrophotometer (Perkin-Elmer) was used to analyze the samples. Kathon CG[®] was quantified by high-performance liquid chromatography (HPLC) us-

ing an LC-6A Shimadzu HPLC, equipped with a C-R3A Chromatopac integrator, an SPD-2A UV-VIS spectrophotometric detector, and an RP-C18 column (particle diameter 5 µm). Shear deformation of emulsions and oil viscosity were determined using a rotational viscometer (model DV-II, Brookfield) with a small adapter chamber, SC 21-29. An Ultra Turrax[®] T25 basic (Janke and Hunkel-IKA-Labortechnik), a Silverson SL 2 homogenizer (Silverson Machines Ltd), and a DLS stirrer (Velp) were used to prepare the emulsions. An Eppendorf 5417 centrifuge was used for the stability tests on the emulsions. pH measurements were performed with an HI 9321 microprocessor pH meter (Hanna Instruments). Microscopic analysis used a Labovert Leitz optical microscope equipped with a Wild MPS 46 Fotoautomat camera. Irradiation tests were performed in Pyrex glass cells (5-ml solutions) under solarboxes equipped with a UVB TL 40/12 RST40T12 lamp (Philips[®]), a UVA TL K0540 W lamp (Philips[®]), and an Osram Ultravitalux lamp (solar spectrum).

METHODS

In order to make a qualitative evaluation of hemp-seed oil and test the validity of the analytical methods adopted, comparative studies were carried out on hemp-seed, olive, and extra-virgin olive oils simultaneously (4,8–11). Two different hemp-seed oil samples, obtained from the same batch of seeds, picked in 1998 and crushed in 1998 and in 1999, were also analyzed. The two hemp-seed oil samples had been produced by two different oil mills, adopting two different seed-crushing techniques. The hemp-seed oil produced in 1998 was obtained by mechanical extraction employing a screw press provided at its extremity with a filter to remove suspended impurities from the oil. The hemp-seed oil produced in 1999 was obtained by mechanical extraction with a method known as "Baglioni": hemp seeds are placed under a millstone with hazelnut shells, which, increasing the contact surface, reduce the crushing time required.

DETERMINATION OF DENSITY

Oil density was determined at 20°C by weighing an exact volume (5 ml) of oil; the determination was carried out three times for each oil sample. The density of oil (ρ_{20} , expressed in g/cm³) is the ratio of weight to volume at 20°C, while the relative density (d^{20}_{4}) is the ratio of the density of oil to the density of water at 4°C. Results were expressed as relative density d^{20}_{4} .

DETERMINATION OF VISCOSITY

Determination with rotational viscometer. The apparent viscosity of oil samples was determined with a Brookfield[®] rotational viscometer employing a small adapter chamber, SC 21, at 25°C. The determinations were performed at 18.6 s⁻¹ on 8 g of oil and were repeated three times per sample.

Determination with capillary viscometer. Kinematic viscosity was determined with an Ostwald capillary viscometer by measuring oil-flow time through a capillary. The kinematic viscosity (ν , expressed in cm²s⁻¹) was calculated from equation 1, whereas the

dynamic viscosity (η , expressed in mPa s) was calculated from equation 2. The measurements were repeated three times for each oil sample and were conducted at 25°C.

$$\nu = Kt \tag{1}$$

$$\eta = K\rho t \tag{2}$$

where K is the viscometer constant (0.01053), ρ is the oil density, and t is the oil flow time through the capillary.

DETERMINATION OF REFRACTIVE INDEX (RI)

The refractive index is an important parameter in terms of quality; it can reveal adulteration if its value is outside the accepted range (1.4672–1.4679). The refractive index was determined with an Abbie refractometer: the oil was placed in the prism cell, whose refractive index was known. The index was determined directly by reading it from the scale.

DETERMINATION OF ACIDITY INDEX

The acidity index l_A is the amount of KOH, in milligrams, required to neutralize the free fatty acids present in 1 gram of fat. It is considered an important analytic parameter, as it indicates the state of conservation of a fat and its quality. This is because the presence of free acids in a fat increases as triglycerides become hydrolyzed, and this process reduces quality. Acidity may also be expressed as the percentage content of oleic acid.

The acidity index was determined as follows: 5 g (m_1) of oil was dissolved in 25 ml of a mixture of absolute ethyl alcohol and diethyl ether in equal volumes, previously neutralized with a solution of 0.1M KOH, using 0.5 ml of phenolphthalein (R_1) as indicator. The dissolved oil was then titrated, adding n_1 ml of KOH 0.1 M until the pink color of phenolphthalein persisted for at least 15 seconds. The acidity index was calculated from equation 3.

One milliliter of KOH N/10 corresponds to 0.0282 g of oleic acid; thus the percentage of oleic acid was calculated from equation 4.

$$I_A = \frac{5.610n_i}{m_1}$$
(3)

Oleic acid % =
$$100 \times \frac{0.0282 \times n}{m_1}$$
 (4)

DETERMINATION OF PEROXIDE NUMBER

Peroxides are the primary products of fat lipoperoxidation; hence determination of the amount of peroxide present in an oil is another analytical method to evaluate its quality. Peroxides are not only oxidizing agents; they also promote the release of iodine from

potassium iodide, and for this reason the peroxide number can be determined by iodometric titration. The peroxide number is the number of active oxygen milliequivalents present in 1000 g of fat mass, which correspond to the milliequivalents of iodine released from potassium iodide titrated with sodium thiosulphate solution.

Degraded oils may have peroxide numbers well below expectations, since the first step of lipoperoxidation, auto-oxidation, may already be completed, and all the hydroperoxides derived from this step may have been transformed into secondary volatile unpleasant-smelling compounds. The peroxide number was determined as follows: Five grams (m_2) of oil was placed in a 250-ml Erlenmeyer flask, and the flask was closed with an emery cap. Thirty milliliters of chloroform/acetic acid (2:3) mixture was then added to the oil sample under stirring. After complete dissolution, 0.5 ml of potassium iodide saturated solution (R₂) was added. The solution was stirred for one minute, and 30 ml of distilled water was added. The mixture was then titrated with 0.01 M sodium thiosulphate solution, added slowly under continuous stirring until the yellow color disappeared. Five milliliters of starch indicator was then added and a dark blue color appeared. The titration continued during addition of sodium thiosulphate solution and vigorous stirring until the blue color disappeared (n_2 ml of 0.01M sodium thiosulphate solution). A control titration was carried out on a reference sample under the same conditions. The final volume $(n_3 \text{ ml of } 0.01 \text{ M sodium thiosulphate solution})$ employed for the control titration must be less than 0.1 ml. The peroxide number, expressed in milliequivalents of oxygen per 1000 grams of fat, is calculated from the following equation:

Peroxide number =
$$\frac{10(n_2 - n_3)}{m_2}$$
 (5)

DETERMINATION OF CONJUGATED DIENES AND TRIENES

Spectrophotometric examination can provide information on the quality of a fat, its state of conservation, and any changes produced in it by technological processes. Absorption at the wavelength 232 nm is due to the presence of conjugated diene systems, while trienes absorb at 262, 268, and 274 nm. The normal spectrum of a non-rancid virgin oil shows no absorption at these wavelengths. Oil was dissolved in 1-butanol, spectrophotometrically pure in the range of wavelengths considered. Extinctions at the various wavelengths were then detected with reference to pure solvent. The absorbence values were expressed as specific extinctions $E^{1\%}_{1 \text{ cm}}$ (the extinction of 1% solution of the fat in the specified solvent, at a thickness of 1 cm), conventionally indicated by *K*, also referred to as the "extinction coefficient." In accordance with the official method in the EEC regulations, spectrophotometric analysis of oil involves determining the specific extinction at a wavelength of 232 nm and determining the variation in specific extinction, which is given by the following equation:

$$\Delta K = K_{262} - \frac{K_{262} + K_{274}}{2} \tag{6}$$

For non-rancid virgin oils, ΔK is below 0.010.

Pure oil analysis. To simulate the degradation of oil over time, an accelerated stability test was performed: spectrophotometric analysis was carried out on oil samples before and after heating to 40°C for 24 hours.

Oil-in-emulsion analysis. To evaluate the antioxidant effect of excipients, namely Sepigel 305[®] and Carbopol 1382[®], on the oil dispersed in an O/W emulsion, simple formulations containing these two products were prepared, (Table I).

Prior to spectrophotometric examination the oil had to be extracted from the emulsion. It was thus necessary to break the system by adding NaCl crystals under stirring. The dispersion was then subjected to centrifugation at 13000 rpm for 10 minutes to separate the oil from the water phase. The fat supernatant phase was collected and analyzed spectrophotometrically.

The procedure employed to prepare the oil samples was as follows: A 0.1% oil-in-1-butanol solution was prepared, placing 27 µl of oil (oil density being 0.92 g/ml) in a 25-ml volumetric flask and diluting it to volume with 1-butanol. The solution was stirred on vortex and subjected to spectrophotometric analysis. To calculate ΔK the absorbance values (E_{λ}) detected by the spectrophotometer were converted into specific extinctions ($E^{1\%}_{1 \text{ cm}}$ or K) from the following equation:

$$K_{\lambda} = E_{\lambda}/C \tag{7}$$

where C is the solution concentration expressed in g/100 ml.

IRRADIATION TEST

Spectrophotometric determination of conjugated dienes and trienes is also useful to evaluate the photostability of an oil. As a consequence of photodegradation, the number of conjugated systems considerably increases.

In order to evaluate photostability, oil samples were irradiated under UVB lamps. Conjugated dienes and trienes were determined after two hours' irradiation, as described above. Three grams of oil were placed in Pyrex containers with caps and irradiated for two hours under magnetic stirring, at 10 cm from the lamp. The mean surface area exposed to radiation was 10.17 cm². The radiation intensity of the lamp was measured with a CO.FO.ME.GRA measurer with a probe sensitive to radiation in the range of 290–400 nm. The radiation intensity recorded for the UVB lamp was 2.6×10^{-4} W cm⁻²; this is double the intensity of UVB solar radiation (1.3×10^{-4} W cm⁻²) (12). Two variables were taken into account: the cap on the Pyrex containers might act as a

Table I Composition of Formulations			
Ingredients	Emulsion 1 (w/w %)	Emulsion 2 (w/w %)	
Vegetable (hemp-seed, olive) oil	5	5	
Sepigel 305®	1		
Carbopol 1382 [®]		0.25	
NaOH 10% in water		0.27	
Water	94	94.98	

radiation screen, and during emission the lamp might dissipate thermal energy, thereby heating the samples. Both phenomena can influence the formation of conjugated dienes and trienes. Thus, to evaluate the method qualitatively, the photostability study was done in three different ways:

1. The capped Pyrex containers were placed in a closed solar box.

2. The open Pyrex containers were placed in a closed solar box.

3. The capped Pyrex containers were placed in an open solar box, to dissipate thermal energy from the lamp.

MALONDIALDEHYDE AND MONOALDEHYDE DETERMINATION (11)

Malondialdehyde (MDA) and monoaldehyde (MONO) are formed from oxidative reactions of unsaturated fatty acids followed by the decomposition of tryglicerides; they produce an unpleasant smell.

The presence of MDA and MONO, an indication of rancidity, could be revealed through colorimetry with thiobarbituric acid (TBA). TBA reacts with the two aldehydes to give two colors that can easily be determined spectrophotometrically. To detect the presence of MDA and MONO in the oils, samples were prepared as follows: an aqueous solution (100 ml) was prepared containing 15% w/w of trichloroacetic acid (TCA), 0.375% w/w of thiobarbituric acid (TBA) in hydrochloric acid (0.25 mol/l), and 18.8 µl of oil. The solution was placed in a thermostatic water bath (at 100°C), incubated for 15 minutes, and then transferred to an ice bath. Three milliliters of 1-butanol were added under stirring; the solution was then transferred to a separating funnel to extract any pink or yellow coloration that had formed from the reaction of TBA with the aldehydes. The reactions are:

OHC-CH₂-CHO + TBA → TBA-MDA-TBA (pink) R-CH₂-CHO + TBA → TBA-MONO (yellow)

The supernatant organic phase was extracted and analyzed spectrophotometrically at 535 nm and 450 nm to detect, respectively, the pink and the yellow coloration.

DETERMINATION OF CHLOROPHYLL

Chlorophyll is a lipophilic yellowish-green pigment present in the non-saponifiable matter of an oil; it influences taste and smell and determines color. It is a relatively unstable molecule: it easily degrades under the influence of light and heat.

To quantify the chlorophyll content in hemp-seed oil, a calibration curve was made against a reference pigment (a standard solution of 4.0% chlorophyll in maize germ oil). Non-pigmented maize germ oil was used both as a reference for spectrophotometric analysis and as a solvent for dilutions. Three dilutions of the standard pigmented solution were prepared, containing, respectively, 0.0016%, 0.008%, and 0.004% chlorophyll. The three standard solutions were analyzed spectrophotometrically in the 300–600 nm range. The same procedure was applied to the 1999 hemp-seed oil and extravirgin olive oil samples to determine their percentage chlorophyll content.

EVALUATION OF THE PHOTOSTABILITY OF CHLOROPHYLL

Chlorophyll is a photosensitive molecule that may undergo structural changes on exposure to light, possibly influencing lipoperoxidation. The formation of conjugated double bonds may be accelerated under the influence of free radicals derived from the photodegradation of chlorophyll. We thus evaluated its photostability by irradiating hemp-seed and extra-virgin olive oils under UVA and under visible light for increasing durations of time. At each scheduled time the irradiated oil samples were examined spectrophotometrically. Oil samples were placed in Pyrex containers 10 cm from the UVA or OSRAM (solar spectrum) lamp. Spectrophotometric analysis was done after 30, 90, and 180 minutes of irradiation, as described above.

The radiation intensity emitted by the UVA lamp was measured, as for the UVB lamp, and was 1.89×10^{-4} W cm⁻². Standard solar radiation intensity is 1.1×10^{-3} W cm⁻² (12), slightly above that of the UVA lamp used.

DETERMINATION OF VITAMIN E IN HEMPOIL

Vitamin E (tocopherol) is a lipophilic compound contained in small amounts in the non-saponifiable matter of oils. Tocopherols are efficient antioxidants if present in the optimal ratio versus PUFAs (the tocopherol/fatty acid ratio should be above 0.8). Vitamin E content was determined by HPLC under the following conditions:

- Column: Supersphere 100-RP 18 (length 5 cm, internal diameter 4.6 cm)
- Eluent: ethanol/water: 92/8
- Flow: 1.2 ml/min
- Detector: UV (λ = 295 nm)
- Retention time: 12 min

Two samples of the 1998 hemp-seed oil and two of the 1999 batch were dispersed in methanol at a 1:2 ratio, stirred on vortex, and centrifuged at 6000 rpm for 10 minutes to promote passage of tocopherols into the alcoholic phase.

A calibration curve versus the standard reference substance (α -tocopherol) was made to determine concentration. Standard solutions containing three different concentrations of α -tocopherol were analyzed by HPLC under the above conditions. The calibration curve was linear, indicating low standard error and high correlation coefficients within the concentration range. Oil samples were then analyzed by HPLC under the above conditions.

FORMULATIVE STUDIES

DETERMINATION OF APPARENT VISCOSITY

The viscosity of the emulsions was determined at $25^{\circ}C \pm 0.1^{\circ}C$ using a Brookfield viscometer with a small SC 029 adapter chamber. The determinations were performed at 5 s⁻¹ shear stress on 13 g of each sample.

RHEOLOGICAL RUNS (13-15)

In order to reveal any structuring or destructuring of the systems, flux rheograms were determined on 13 g of each sample, in steady-state conditions at $25^{\circ}C \pm 0.1^{\circ}C$, using a Brookfield viscometer with a small SC 029 adapter chamber. Shear stress values were determined at increasing and decreasing shear rates, with each shear rate maintained for one minute.

pH DETERMINATION

pH values were determined on each sample at room temperature before and after heating to 40° C for 24 h.

CENTRIFUGE TEST

An accelerated test of emulsion stability was performed by centrifuging a small amount of each sample in an Eppendorf[®] centrifuge at 3000 and 4000 rpm, checking for outcrop or phase separation every 10 minutes.

PREPARATION OF EYE GEL-EMULSION WITH VEGETABLE OILS

The emulsion was prepared employing (a) as lipid, hemp-seed, olive, or extra-virgin olive oils and (b) as rheological modifier with emulsifier activity, Carbopol 1382[®] (acrylates/C10-30 alkyl acrylate crosspolymer) neutralized with a 10% NaOH aqueous solution. The polymer was dispersed in the aqueous phase, heated to 60°C under stirring with Ultra Turrax T 25 homogenizer. The lipid phase was then added to the aqueous phase, homogenizing until the system reached room temperature. An aliquot (0.272%) of 10% NaOH aqueous solution was added to the emulsion until it became homogeneous.

PREPARATION OF SPRAYABLE AFTER-SUN FLUID CREAMS (MILK) WITH VEGETABLE OILS

Recent research has focused on sprayable cosmetic products, whose functional and sensory characteristics meet market demands. These products are generally emulsions with high viscosity, though they are sprayable using a device intended for liquids. Fluid sprayable creams meet consumer demands for cosmetics that are easy to apply. The emulsions were prepared employing, as lipid, the hemp-seed, olive, or extra-virgin olive oils and, as rheological modifier, Pemulen TR-1[®] (acrylates/C10-30 alkyl acrylate crosspolymer) neutralized with 18% NaOH aqueous solution. The emulsifiers employed in their preparation were Polysorbate 20 (Tween 20[®]), Polysorbate 40 (Tween 40[®]), Peg-40-hydrogenated castor oil (Cremophor RH®40), methyl glucose sesquistearate (20) OE (Glucamate SS E20®), and LRI® solubilizer. The gels were initially obtained by dispersing the polymer in cold water and homogenizing with Ultra Turrax T25 for about one minute. The system was then neutralized by adding an aqueous solution of NaOH under mild stirring. O/W emulsions were subsequently obtained by slowly adding the emulsifiers and oils to the aqueous phase under homogenization. The sprayable emulsions were then characterized through pH determination, test of centrifugation, and studies of viscosity and rheological behavior, following the methods described above. Some

preliminary runs were carried out to obtain a sprayable O/W emulsion whose pH was skin-compatible and that had an acceptable consistency and texture. The emulsion was modified by reducing the amount of Pemulen TR-1 and of 18% NaOH aqueous solution, as the initial cream was too thick and had a basic pH. NaOH aqueous solution was added slowly, and the pH was monitored to determine the minimum amount necessary to neutralize the system. This emulsion had acceptable rheological behavior (pseudoplastic with no hysteresis areas) and acceptable viscosity and pH. It was therefore chosen as the basic formulation from which to prepare some systems. Each emulsion was prepared with both hemp-seed and olive oils. The systems were characterized by evaluating their stability, pH, apparent viscosity (at 5 s⁻¹), and rheological behavior, as described above. Each emulsion was then centrifuged at 3000 rpm for 60 minutes, and checked for any outcrop or phase separation every 10 minutes. The test was repeated at 4000 rpm. To verify the sprayability of the emulsions, an aliquot of each formulation was placed in a pump supply device. The product was then sprayed onto a sheet of glass, and uniformity of distribution was checked visually. All emulsions were also examined through an optical microscope.

PREPARATION OF EMULSIONS WITH VEGETABLE OILS FOR USE AS FACIAL MOISTURIZING CREAMS

These emulsions were obtained after a series of systems had been tested to determine the best systems. The best emulsions were obtained with the following emulsifiers: PEG-4-olivate (Olivem 700[®]), cetearyl alcohol/cetearyl glucoside (Montanov 68EC[®]), C 14-22 alkylalcohol/C 12-20 alkylglucoside (Montanov L[®]), polyglyceryl-3-methylglucose distearate (Tego Care[®]), sucrose tristearate or palmitate (sucroesters DUB SE[®] 3S and 15P), and sucrose mono (or di)stearate (or palmitate) (sucroesters SP[®] 30 and 50). These systems were characterized with the same methods previously described.

Face cream 9. This emulsion was prepared with the cold-hot technique: the lipid phase (Olivem 700[®], dimethicone, hemp-seed and olive oils, Finsolv TN[®], cetearyl alcohol and glyceryl monostearate with the addition of Eurol BT[®]) was heated under mild stirring until complete homogenization. The aqueous phase (hydrolyzed milk protein, *Hamamelis* distillate, and filtered water) was prepared and slowly added to the hot lipid phase under homogenization at 11000 rpm. The homogenization was continued until the correct viscosity was achieved. The emulsion was cooled to room temperature under mechanical stirring at 100 rpm. Ten milliliters of an aqueous solution of Kathon CG[®] and Gram 1[®] were added to the emulsion at 25–30°C. Sepigel 305[®] was added, as rheological modifier, at room temperature, homogenizing at 11000 rpm for a few minutes. An alternative to this face cream was produced by adding Proteol OAT[®] as secondary emulsifier (Face cream 9B).

Face cream 10. The emulsion was prepared with the hot technique. The lipid phase (Montanov $68EC^{\ensuremath{\mathbb{R}}}$ or Montanov $L^{\ensuremath{\mathbb{R}}}$, dimethicone, hemp-seed and olive oils, Finsolv TN[®], and Eurol BT[®] was heated to 80°C, under mild stirring until uniformity was achieved. The aqueous phase (*Hamamelis* distillate and filtered water) was heated to 80°C and slowly added to the aqueous phase, homogenizing by Ultra Turrax T25 at 11000 rpm. The homogenization continued until the desired viscosity was achieved. The emulsion was cooled to room temperature, while being stirred mechanically at 100 rpm. Gram 1[®], Kathon CG[®], and Sepigel 305[®] were added at 25°C as for face cream 9.

Face cream 11. The lipid phase (Tego Care, dimethicone, hemp-seed and olive oils,

Finsolv TN[®], and Eurol BT[®]) was completely melted at about 50°C. The aqueous phase (*Hamamelis* distillate and filtered water) was heated to 50°C. The hot aqueous phase was then slowly added to the lipid phase under homogenization, the emulsion was cooled, and the preservatives added. Sepigel 305[®] was added to the emulsion to increase its viscosity. This formulation could also be used as a fluid body cream, omitting the Sepigel 305[®].

Face cream 12. The emulsion was prepared using, as emulsifiers, sucrose stearate or palmitate; some runs were first made to determine the correct percentage of the emulsifiers and the best preparation technique. In the first run the two emulsifiers were both dispersed in the lipid phase, but the result was not satisfactory. The more lipophilic sucroester (DUB SE[®] 3S) was thus dispersed in the lipid phase while the more hydrophilic one (DUB SE[®] 15P) was dispersed in the aqueous phase. This adjustment produced a homogeneous system with optimal viscosity.

Following this method, the emulsifier DUB SE[®] 15P and *Hamamelis* distillate were dispersed in warm water. The mixture was homogenized at 11000 rpm for one minute. The lipid phase (DUB SE[®] 3S, cetearyl alcohol, Finsolv TN[®], olive oil, hemp-seed oil, dimethicone, and Eurol BT[®]) was slowly added to the aqueous phase under homogenization. The system was then cooled under stirring, and Kathon CG[®], Gram 1[®], and Sepigel 305[®] were added to the cold emulsion as described above.

Face cream 13. Emulsion 13 was obtained employing the sucroesters SP 30 and SP 50 from Sisterna as emulsifiers; these and the *Hamamelis* distilled water were added to filtered water at 70°C, homogenizing the mixture at 11000 rpm for one minute. The lipid phase (Finsolv TN[®], hemp-seed oil, olive oil, dimethicone, Eurol BT[®], and cetearyl alcohol), at about 65°C, was slowly added to the aqueous phase under homogenization, and cooled under mechanical stirring at 100 rpm. The preservatives and Sepigel 305[®] were added to the system at room temperature, homogenizing until the desired consistency was achieved.

PERCUTANEOUS PENETRATION IN VITRO OF KATHON CG® FROM AN O/W EMULSION THROUGH PIG SKIN (16)

Kathon CG®, a common preservative contained in many commercial formulations, was also present in all the emulsions prepared in this study. The compound possesses both antibacterial and antifungal properties and may also induce allergic reactions (17). Mutagenic activity has also been reported (18) on bacteria treated with a number of cosmetic products listing methylisothiazolinone and methylchloroisothiazolinone, the components of Kathon CG®, among their ingredients. Thus the release of this biocide from face cream 11 was investigated, and likewise the skin absorption of Kathon CG[®], so as to determine the amount that crosses the skin and to evaluate any risk. Skin penetration studies play an essential role in optimizing formulation design for dermal and transdermal delivery. Experimental use of in vitro permeation techniques, such as Franz-type diffusion cells, is therefore very important. This system enables the kinetics of the uptake of different components and their diffusion from pharmaceutical or cosmetic formulations (emulsions, gels, or creams) to be evaluated. The concentrations of such components in the deeper skin layers may also be determined by analyzing the receptor fluid. The Franz-cell system is widely used, being inexpensive, quick, and reproducible (16,19). Pig skin is suitable for *in vitro* dermal penetration studies, and the

anatomic similarity between pig and human skin makes the results obtained with this technique predictive for human skin absorption.

The pig skin was prepared: A fresh pig's ear was thoroughly washed in physiological solution and the bristles removed. The skin was then fixed onto a cork mat and the external surface of the ear cut away. To make the conditions reproducible, skin strips of equal thickness were cut, consisting of horny layer, epidermis, and a part of the dermis; there is little or no subcutaneous fat in the edge of the ear. Disks of the same diameter as the Franz cells (2.5 cm^2) were cut from these strips.

The emulsion was applied to the skin disk held in place between the donor and receptor compartments of the Franz-static diffusion cell; the epidermis was in contact with the donor compartment and the dermal layer in contact with the receptor compartment, consisting of physiological solution (0.9% NaCl) under continuous magnetic stirring.

To quantify the amount of Kathon CG[®] that had permeated through the skin, samples were taken every 60 minutes. After 24 hours, the receptor compartment was emptied and replaced with fresh physiological solution; the solution removed was subjected to HPLC analysis, under the above conditions. At the end of 24 hours, the skin was cleaned, cut into small pieces, and placed in a beaker containing the eluent employed for the HPLC analyses, in order to determine any Kathon CG[®] trapped in the tissues. Each sample was injected into the HPLC apparatus following the procedures reported below. The experiment was repeated twice.

The concentration of Kathon $CG^{\textcircled{B}}$ in the receptor solution was found directly from the standard calibration graph obtained by injecting standard solutions of Kathon $CG^{\textcircled{B}}$ at different concentrations, between 1 mg/l and 6 mg/l, into the HPLC apparatus. The analytical conditions were as follows:

- Column: Cromasil C 18
- Eluent: methanol/water: 30/70
- Flow: 0.7 ml/min
- Detector: UV (λ = 275 nm)
- Retention time: 7.4 min

The calibration curve showed a good linear correlation between peak areas and sample concentration ($R^2 = 0.9978$).

The results of the study of permeation showed that only small amounts of the preservative penetrated the dermis: after 24 hours the concentration of Kathon CG[®] in the receptor phase was negligible.

RESULTS AND DISCUSSION

QUALITY INDICES AND PHYSICOCHEMICAL CHARACTERISTICS

The quality indices and physicochemical characteristics of the hemp-seed oils (1998 and 1999) and olive oil are in Table II.

ACIDITY

The acidity indices and percentages of oleic acid are listed in Table III. Both batches of

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	0				
Parameters	Hemp-seed oil (1998)	Hemp-seed oil (1999)	Olive oil	Extra-virgin olive oil	
Relative density (d ²⁰ ₄)	0.925	0.924	0.916		
Rotational viscosity at 18.6 s^{-1} (mPa s)	40	37.5	60	55	
Capillary viscosity (mPa s)	40.420				
Refractive index (RI)	1.4735	1.4760	1.4665	1.4665	

 Table II

 Physicochemical Characteristics of the Vegetable Oils Considered

Table III

Acidity Indices and	Percentage of G	Oleic Acid	in the Vegetal	ole Oils (Considered

	Hemp-seed oil (1998)	Hemp-seed oil (1999)	Olive oil
IA	3.85 (±0.46)	14.10 (±1.31)	0.44 (±0.02)
Oleic acid (%)	1.93 (±0.18)	7.13 (±0.95)	0.22 (±0.01)

Values in parentheses are standard deviations, determined on four samples.

 I_A = aliquot (in mg) of KOH 0.1M necessary to neutralize the fatty acids that are free in 1 gram of fat mass.

hemp-seed oil (1998 and 1999) had free acidity above 1%, the upper limit for the "extra-virgin" category (EEC Regulations), while the acidity of olive oil was below the limit. Hemp-seed oil (1999) had a significantly higher free-fatty-acid content, although it was younger, probably due to the different method (Baglioni) employed for crushing the seeds. The acidity index of the commercial olive oil was in agreement with that declared on the label.

PEROXIDE NUMBER

Peroxide numbers, expressed as milliequivalents of O_2 per 1000 grams of fat mass, were found to be 5.15 for the 1998 hemp-seed oil and 1.57 for the 1999 hemp-seed oil. Both kinds of oil had peroxide values below the limit of 10 established for "extra virgin" oil. Moreover, the peroxide numbers of both hemp oils were lower than 20, the threshold limit for edible oils.

CONJUGATED DIENES AND TRIENES

Results of UV characterization are reported in Table IV. Absorptivity differences at 232

Table IV K_{232} and ΔK Values of the Oils Considered, Dissolved in 0.1% 1-Butanol					
	Hemp-seed oil (1998)	Hemp-seed oil (1998) (after 24 h at 40°C)	Hemp-seed oil (1999)	Olive oil	Extra-virgin olive oil
$K_{232} \Delta K$	2.58 (±0.23) 0.025 (±0.003)	2.66 (±0.11) 0.030 (±0.001)	2.12 (±0.12) 0.025 (±0.001)	2.72 (±0.08) 0.075 (±0.002)	2.20 (±0.09) 0.0

Values in parentheses are standard deviations, determined on four samples.

nm and at 262, 268, and 274 nm (ΔK) were used to measure the increase in conjugated dienes and trienes, respectively.

Both for the two kinds of hemp-seed oil and the commercial olive oil, K_{232} and ΔK values were above the limits set for "extra virgin" oils. The absorptivity (0.1% oil in 1-butanol) at 232 nm and the ΔK value increased with temperature, as is shown for the hemp-seed oil (1998), heated for 24 hours at 40°C.

Tables V and VI report the results of the spectrophotometric analysis carried out to evaluate whether the polymeric vehicles Sepigel 305[®] and Carbopol 1382[®] enhance the formation of conjugated dienes and trienes in vegetable oils in which they are dispersed. The combined results (Tables V and VI) show that both hemp-seed oils and the commercial olive oil had much higher oxidative stability when dispersed in the Sepigel 305[®] than in the Carbopol 1382[®] system.

IRRADIATION TEST

Table VII shows the results of the irradiation test. Olive oil was much more photostable

	UV-Visible Char	acterization of Oils	Table V Dispersed in Sepigel	305 [®] System (emu	lsion 1)
	Hemp-seed oil (1998) (before heating)	Hemp-seed oil (1998) (after 24 h at 40°C)	Hemp-seed oil (1999) (after 24 h at 40°C)	Olive oil (after 24 h at 40°C)	Extra-virgin olive oil (after 24 h at 40°C)
$\frac{K_{232}}{\Delta K}$	2.80 (±0.21) 0.015 (±0.003)	4.45 (±0.18) 0.020 (±0.001)	4.23 (±0.10) 0.020 (±0.004)	3.51 (±0.14) 0.020 (±0.003)	3.51 (±0.09) 0.020 (±0.003)

Values in parentheses are standard deviations, determined on four samples.

Table VI UV-Visible Characterization of Oils Dispersed in Carbopol 1382 [®] System (emulsion 2)					ulsion 2)
	Hemp-seed oil (1998) (before heating)	Hemp-seed oil (1998) (after 24 h at 40°C)	Hemp-seed oil (1999) (after 24 h at 40°C)	Olive oil (after 24 h at 40°C)	Extra-virgin olive oil (after 24 h at 40°C)
$K_{232} \Delta K$	2.76 (±0.13) 0.010 (±0.001)	2.90 (±0.16) 0.015 (±0.002)	4.04 (±0.18) 0.030 (±0.006)	4.04 (±0.23) 0.030 (±0.006)	2.55 (±0.17) 0.010 (±0.004)

Values in parentheses are standard deviations, determined on four samples.

Table VII
K_{232} and ΔK Values of the Vegetable Oils after 2 Hours of UVB Irradiation

	Hemp-seed oil (1998)	Hemp-seed oil (1999)	Olive oil
<i>K</i> ₂₃₂	5.54 (±0.11)	8.92 (±0.30)	3.07 (±022)
ΔK	0.015 (±0.001)	0.035 (±0.005)	0.095 (±0.002)

Values in parentheses are standard deviations, determined on four samples.

under UVB irradiation than the hemp-seed oils. The 1998 hemp-seed oil had higher photostability under UVB irradiation than the 1999 oil.

MALONDIALDEHYDE (MDA) AND MONOALDEHYDE (MONO)

Oil samples subjected to thiobarbituric acid assay were opportunity-treated to extract the colors, pink for MDA or yellow for MONO. The absorptivites of the prepared samples were measured at 450 and 535 nm for the MONOs and the MDAs, respectively. The results of the colorimetric analyses are in Table VIII. The 1999 hemp-seed oil had a higher peroxidation level than the 1998 oil. Contrary to expectations, the commercial olive oil also had a significant peroxidation level.

CHLOROPHYLL

The chlorophyll concentration observed in the hemp-seed oil was 0.0036% of the fat mass, while the extra-virgin olive oil had a lower chlorophyll content, only 0.0024%. Figure 1 shows an example of a UV-visible spectrum of hemp-seed oil (1998), diluted 1:2 in maize-germ oil.

CHLOROPHYLL PHOTOSTABILITY

Pigments like chlorophyll are also involved in auto-oxidation and photo-oxidation mechanisms. For this reason, spectrophotometric determinations at 416 nm and 671 nm, before and after irradiation, were carried out. Figure 2 shows the results of spectrophotometric determinations at 416 nm and 671 nm, after UVA irradiation.

The chlorophyll in the extra-virgin olive oil had a higher photostability than that in the 1998 hemp-seed oil, which degraded faster under UVA irradiation than under visible irradiation (see Figure 3). This is probably due to the composition of olive oil, which contains many more anti-oxidant components (such as BHT and tocopherols) that could protect the pigment against photodegradation.

VITAMIN E IN HEMPOIL

Qualitative tocopherol composition was determined in both hemp-seed oils (1998 and 1999). For the 1998 hemp-seed oil, the concentration of tocopherol was $8.2 \cdot 10^{-6}$ g/100 ml, and for the 1999 oil it was $2.5 \cdot 10^{-5}$ g/100 ml. The quantitative results obtained are reasonable when one considers the progressive degradation of this vitamin

	Absorptivites at 450 n	Fable VIII m and at 535 nm of Oil Samples	
	Hemp-seed oil (1998)	Hemp-seed oil (1999)	Olive oil
K ₄₅₀	0.063 (±0.09)	0.075 (±0.07)	0.130 (±0.13)
K ₅₃₅	0.041 (±0.05)	0.048 (±0.08)	0.087 (±0.03)

Values in parentheses are standard deviations, determined on four samples.



Figure 1. UV-visible spectrum of hemp seed oil (1998), diluted 1:2 in maize-germ oil.

in oil samples during storage, especially for the 1998 oil. In consideration of this satisfactory performance, we chose the 1998 hemp-seed oil and the olive oil as lipids to formulate emulsions, which were then characterized by stability and rheological profile.

FORMULATIVE STUDIES

EYE GEL-EMULSION 3 (Table IX)

The viscosity range (Table X) denoted that the O/W emulsions prepared were fairly consistent and quite stable upon heating. Their pH values (Table X) were skin-compatible. The rheological study of the eye gel-emulsions showed a pseudoplastic behavior with a high yield value for all three oils employed. This behavior is typical of gel systems that are normally highly structured. The gel-emulsion containing extra-virgin olive oil showed a small hysteresis area and a thixotropic trend.



Figure 2. Study of the photostability of the chlorophyll contained in hemp-seed and olive oils under UVA irradiation.



Figure 3. Comparative study of the degradation of the chlorophyll contained in hemp-seed and olive oils under UVA and visible irradiation.

Table IX	
Formulation of Eye Gel-Emulsion 3 Prepared v	with Hemp-Seed Oil and Olive Oil

Ingredients	Percentage (% p/p)
Vegetable (hemp-seed or olive) oil	5
Carbopol 1382®	0.25
NaOH 10%	0.272
Water	94.478

	pH and Viscosity, Indicative of the Influence of the Vegetable Oils on the Emulsions, Before and After Heating						
	Eye gel–emulsion with hemp-seed oil (before heating)	Eye gel–emulsion with hemp-seed oil (after 1 h at 40°C)	Eye gel–emulsion with olive oil (before heating)	Eye gel–emulsion with olive oil (after 1 h at 40°C)	Eye gel–emulsion with extra-virgin olive oil (before heating)	Eye gelemulsion with extra-virgin olive oil (after 1 h at 40°C)	
Viscosity at 5 s^{-1} (mPa s)	8150	7500	9400	8550	9800	9800	
рН	4.80	4.75	5.15	5.15	5.10	4.97	

Table X

SPRAYABLE AFTER-SUN MILK

The composition of the basic formulation chosen to prepare the sprayable emulsion was: vegetable oil (10%), 18% NaOH aqueous solution (0.27%), Pemulen TR-1[®] (0.10%), and water (89.63%). Table XI gives the compositions of five different formulations for sprayable after-sun milks; Table XII shows their viscosity and pH characteristics. The viscosity range of these O/W emulsions was typical of fluid systems, and their pH values were skin-compatible.

All the after-sun emulsions considered showed a pseudoplastic trend with no hysteresis area and with a tendency to Newtonian flux. All proved to be sprayable and stable to centrifugation at 3000 rpm. Each of the surfactants employed was compatible with both the hemp-seed and the olive oil, except for the glucamate: emulsion 5 was found to be unstable.

MOISTURIZING FACE CREAM

Several trials were carried before the most suitable formulations were determined. The choice of surfactant and of rheological modifiers greatly influenced the overall quality of the system. Moreover, it was found to be quite difficult to prepare emulsions containing olive oil and/or hemp-seed oil. Tables XIII, XIV, XV, XVI, and XVII report the compositions of some moisturizing face creams incorporating olive oil and hemp-seed oil and employing five different surfactants. These five systems had high stability, suitable subjective feel on the skin, and thixotropic pseudoplastic flux.

CHARACTERIZATION OF FACE CREAMS 9A AND 9B

Emulsions 9A and 9B proved to be stable after centrifugation at 4000 rpm for 10 minutes, both before and after heating (40°C for 24 hours). Table XVIII shows the viscosity and pH values, as the means of three measurements. Both emulsions showed a pseudoplastic behavior with a small hysteresis area. As an example, Figure 4 shows the rheological trend of emulsion 9B before and after heating at 40°C for 24 hours.

Table XI Percentage Composition of Sprayable After-Sun Milks Containing the Oils Studied						
Ingredients	Emulsion 4 (w/w%)	Emulsion 5 (w/w%)	Emulsion 6 (w/w%)	Emulsion 7 (w/w%)	Emulsion 8 (w/w%)	
Vegetable oil	7.0	7.0	7.0	7.0	7.0	
Tween 20 [®]		0.50				
Tween 40 [®]	0.50					
NaOH 18%	0.27	0.27	0.27	0.27	0.27	
Pemulen TR-1®	0.10	0.10	0.20	0.10	0.10	
Cremophor RH40®			0.50			
LRI®				0.60		
Glucamate					0.50	
Methyl glucose dioleate					0.20	
Water	92.13	92.13	92.03	92.03	91.93	

Characterization of the Five Sprayable After-Sum Milks										
	Emulsion 4 with hemp oil	Emulsion 4 with olive oil	Emulsion 5 with hemp oil	Emulsion 5 with olive oil	Emulsion 6 with hemp oil	Emulsion 6 with olive oil	Emulsion 7 with hemp oil	Emulsion 7 with olive oil	Emulsion 8 with hemp oil	Emulsion 8 with olive oil
Viscosity at 5 s ⁻¹ (mPa s)	3000	3150	4850	3150	2550	3600	1650	2950	1200	1600
рН	6.71	7.76	7.05	7.33	7.50	7.9●	7.62	8.03	8.02	8.44

 Table XII

 Characterization of the Five Sprayable After-Sum Mil

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Ingredients	Emulsion 9A (w/w %)	Emulsion 9B (w/w %)
Olivem 700 [®]	6	5
Finsolv TN [®]	4	4
Hemp-seed oil	3	3
Olive oil	3	3
Dimethicone	0.5	0.5
GMS®	1.5	2
Eurol BT [®]	0.1	0.1
Proteol OAT®		1
Hydrolyzed milk protein	1	1
Hamamelis distillate	10	10
Sepigel 305 [®]	0.5	1.7
Kathon CG [®]	0.05	0.05
Gram I®	0.3	0.3
Water	70.05	68.35

 Table XIII

 Composition (w/w %) of Two Formulations (9A and 9B) Containing Olive Oil and Hemp-Seed Oil, Using Olivem 700[®] as Surfactant

Table XIV

Composition (w/w %) of Three Formulations (10A, 10B, and 10C) Containing Olive Oil and Hemp-Seed Oil, Using Montanov[®] as Surfactant

Ingredients	Emulsion 10A (w/w %)	Emulsion 10B (w/w %)	Emulsion 10C (w/w %)
Montanov 68EC®	6	5	
Montanov L [®]		-	5
Finsolv TN [®]	5	5	5
Hemp-seed oil	3	3	3
Olive oil	2	2	2
Dimethicone	0.5	0.5	0.5
GMS®	1	2	1
Eurol BT [®]	0.1	0.1	0.1
Hamamelis distillate	10	10	10
Sepigel 305 [®]	2		2
Gram I®	0.3	0.3	0.3
Kathon CG [®]	0.05	0.05	0.05
Water	70.05	72.05	71.05

Table XV

Composition (w/w %) of Formulation 11 Containing Olive Oil and Hemp-Seed Oil, Using Tego Care® as Surfactant

Ingredients	Emulsion 11 (w/w %)
Tego Care [®]	5
Finsolv TN [®]	5
Hemp-seed oil	3
Olive oil	2
Dimethicone	0.5
Eurol BT®	0.1
Hamamelis distillate	10
Sepigel 305 [®]	0.5
Gram I [®]	0.3
Kathon CG [®]	0.05
Water	73.55

Ingredients	Emulsion 12 (w/w %)
DUB SE [®] 3S	2
Finsolv TN®	5
Hemp-seed oil	3
Olive oil	2
Dimethicone	0.5
Eurol BT [®]	0.1
GMS	2.5
Hamamelis distillate	10
DUB SE [®] 15P	4
Sepigel 305 [®]	2
Gram I [®]	0.3
Kathon CG [®]	0.05
Water	68.55

 Table XVI

 Composition (w/w %) of Formulation 12 Containing Olive Oil and Hemp-Seed Oil, Using Sucrose

 Tristearate or Palmitate (DUB SE® 3S and DUB SE® 15P) as Surfactants (20,21)

Table XVII

Composition (w/w %) of Formulation 13 Containing Olive Oil and Hemp-Seed Oil, Using Sucrose Mono- or Distearate (Sisterna SP30[®] and Sisterna SP50[®]) as Surfactants (15,16)

Ingredients	Emulsion 13(w/w %)
Finsolv TN®	5
Hemp-seed oil	2.5
Olive oil	2.5
Dimethicone	0.5
Eurol BT [®]	0.1
Cetearyl alcohol	2.5
Hamamelis distillate	10
Sisterna SP30®	3
Sisterna SP50®	5
Sepigel 305 [®]	1
Gram I [®]	0.3
Kathon CG®	0.05
Water	68.55

 Table XVIII

 Viscosity and pH Values of Emulsions 9A and 9B

	Emulsion 9A	Emulsion 9B
Viscosity (mPa s) at 1.25 s^{-1}	9000	35400
Viscosity (mPa s) at 2.5 s^{-1}	7600	23500
Viscosity (mPa s) at 5 s^{-1}	6600	14800
pH before heating	5.95	4.02
pH after heating (40°C for 24 h)	5.86	4.05



Figure 4. Rheological trend of emulsion 9B.

CONCLUSIONS

The physicochemical characteristics of hemp-seed oil found were close to those reported in the literature. Hemp-seed oil was stable against oxidation, as shown by the peroxide number below 20 and by the results of MDA and MONO assays. This stability is probably due to the oil's vitamin E content. Emulsions prepared with vegetable oils (olive and hemp-seed oils) were stable and showed a pseudoplastic-Newtonian flux. Some gel-emulsions were obtained that are easily sprayable on the skin and have a pleasant skin feel and texture.

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