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Enhancement of *trans*-resveratrol photostability by encapsulation in lipid microparticles: *in vitro* and *in vivo* studies

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Lipid microparticles (LMs) loaded with the antioxidant polyphenol, *trans*-resveratrol were developed in order to enhance its photostability in topical formulations. The LMs were prepared by the melt emulsification technique, using tristearin as the lipidic material and hydrogenated phosphatidylcholine as the surfactant. The obtained microparticles were characterized by optical microscopy and release studies. The *trans*-resveratrol loading was 10.8% (w/w). Free or microencapsulated *trans*-resveratrol was introduced in model topical formulations (cream and hydrogel) and irradiated with a solar simulator. The light-induced degradation of *trans*-resveratrol was significantly reduced by incorporation into the LMs both in the cream (the *trans*-resveratrol loss decreased from 34.3% to 19.9%) and in the hydrogel (the *trans*-resveratrol decomposition decreased from 15.4% to 9.4%) vehicles. Moreover, the *in vitro* (i.e., antioxidant action) and *in vivo* (i.e., anti-inflammatory action) biological activities of *trans*-resveratrol in the cream preparation were not altered by the encapsulation process.

1. Introduction

The naturally occurring polyphenol *trans*-resveratrol (3,5,4'-trihydroxystilbene) exhibits a remarkable antioxidant activity and has been reported to have a broad range of beneficial pharmacological properties, including anti-inflammatory effects, cancer prevention, neuro- and cardio-protective actions (Baur and Sinclair 2000; Neves et al. 2012; Kasiotis et al. 2013; Zhenghua et al. 2013). Moreover, several studies have shown that the topical application of *trans*-resveratrol inhibits oxidative skin damage, inflammation, microbial infections, skin tumorigenesis and the cutaneous harmful effects induced by sunlight UV radiation (Jang et al. 1997; Aziz et al. 2005; Ndiye et al. 2011). However, the efficacy of *trans*-resveratrol following skin application is hampered by its high photoinstability. In fact, following UV light exposure resveratrol rapidly undergoes isomerization from the more active and common trans form to the less active cis conformation (Montsko et al. 2008; Shi et al. 2008; Sapino et al. 2009; Bonda et al. 2011; Detoni et al. 2012), with some photodecomposition (Montsko et al. 2008). This represents a disadvantage, especially for topical treatment that greatly exposes the formulation to light. Therefore, in order to preserve the biological activity of *trans*-resveratrol, it is desirable to inhibit its photosensitivity. To enhance the stability of *trans*-resveratrol under light exposure, several approaches have been described including complexation with cyclodextrins (Sapino et al. 2009; Allan et al. 2009) and addition of photostabilizers (Bonda et al. 2011). Moreover, encapsulation techniques based on incorporation in liposomes (Sapino et al. 2009; Detoni et al. 2012), yeast cells (Shi et al. 2008) or lipid nanoparticles (Detoni et al. 2012; Carlotti et al. 2012) have also been reported. However, the latter studies exhibited a limitation, namely the effect of the examined carriers on the photochemical behaviour of *trans*-resveratrol has been evaluated under conditions (e.g., resveratrol concentration, type of vehicles and irradiation source) that are not representative of those that apply to the real application of dermatological products (Shi et al. 2008; Sapino et al. 2009; Allan et al. 2009; Carlotti et al. 2012; Detoni et al. 2012).
In the present study, lipid microparticles (LMs) were evaluated as an alternative carrier system for reducing *trans*-resveratrol isomerization/degradation induced by UV radiation. LMs consist of a solid lipid core stabilised by a layer of surfactant molecules on their surface (Jaspart et al. 2005; Scalia et al. 2015). They are based mainly on physiologically compatible and biodegradable constituents, suitable for topical administration. Compared to liposomes and lipid nanoparticles, and due to their micron dimensions, LMs have the advantages of simpler production and characterization methods, higher stability, reduced amounts of surfactant required for their preparation, higher loading and retention capacity (Elgart et al. 201; Scalia et al. 2015). Moreover, their solid matrix protects the incorporated substance from degradation (Jaspart et al. 2005; Scalia et al. 2015).
The present study reports on the preparation and characterization of LMs loaded with *trans*-resveratrol. The influence of microencapsulation on the photostability of the polyphenol was examined after incorporation of the LMs in model formulations (gel and emulsion) suitable for topical application. Moreover, the effect of the microencapsulation process on the *in vitro* (i.e., antioxidant action) and *in vivo* (i.e., anti-inflammatory action) biological activities of *trans*-resveratrol in the cream preparations is reported.

2. Investigations and results

2.1. Microparticle preparation and characterization

For the preparation of the LMs loaded with *trans*-resveratrol, the melt emulsification technique was used since it avoids the use of organic solvents (Jaspart et al. 2005; Scalia et al. 2015). Tristearin was selected as lipid on the basis of a previous study on the development of resveratrol LMs for dermal application (Scalia et al. 2015). Several emulsifiers (hydrogenated phosphatidylcholine, polysorbate 60 and poloxamer 188) were evaluated for the preparation of the LMs in order to achieve high loading, thereby reducing the amount of microparticles required to attain the target drug concentration in the formulation. This is an advantage, especially for topical products, which do not support high amounts of powder components. Increased resveratrol levels were achieved for the LMs produced with hydrogenated phosphatidylcholine or polysorbate 60, the former surfactant was selected for the following experiments due to its biocompatibility.
The resveratrol content of the microparticles based on tristearin and phosphatidylcholine was 10.8±0.3 %, which corresponded to an encapsulation efficiency of 69.7 %. The latter value would

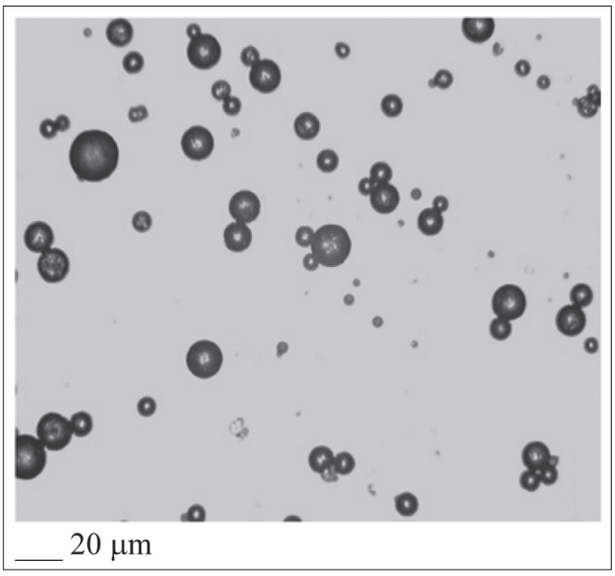

Fig. 1: Optical microscopy image of LMs loaded with *trans*-resveratrol.

include not only the amount of entrapped drug but also the fraction adsorbed on the LM external surface, and therefore represents a process yield rather than a true drug entrapment efficiency.

As illustrated in Fig. 1, investigation by optical microscopy showed a spherical shape for the obtained LMs and the absence of irregular fragments. Moreover, the particle size determined by computerized image analysis, was between 4 and 20 mm (mean diameter, 12.9±5.3 mm) suitable for topical formulations, since particles in this size range are not palpable on application and thus the acceptability of the preparation is not impaired.

Further characterization of the LMs was performed by *in vitro* release studies, using phosphate buffer containing polysorbate 20 as a medium, since resveratrol was sufficiently soluble in it (Scalia et al. 2015) to ensure sink conditions. The release of resveratrol from the LMs was significantly lower (ANOVA and Tukey's post-test) than the dissolution of the plain drug (Fig. 2), which suggested that the polyphenol was incorporated into the particle lipid matrix. The LM release profile exhibited an initial burst release of about 63 % of the incorporated resveratrol, which indicated that a fraction of the polyphenol was adsorbed on the particle surface.

2.2. Photodegradation studies

For the evaluation of the effect of microencapsulation on the photochemical behaviour of *trans*-resveratrol, a hydrophilic cream (oil-in-water emulsion) and a hydrogel were selected as vehicles, because they represent the most common types of dermatological products (Block 2000) and hence simulate real conditions of use. Moreover, in order to minimize possible interactions between the excipients and the polyphenol, basic emulsion and hydrogel formulations were selected. The cream and gel preparations containing *trans*-resveratrol (1%, w/w) in conjunction with unloaded microparticles or equivalent amounts of the polyphenol entrapped in the LMs, were prepared and exposed to the solar simulator.

Following irradiation of the creams, 34.3 % of *trans*-resveratrol was lost in the formulation containing the polyphenol in combination with blank LMs. Incorporation of the polyphenol in the LMs significantly reduced its light-induced degradation in the emulsion vehicle to 19.9 % (Fig. 3). Moreover, as illustrated in Fig. 3, the extent of *trans*-resveratrol photodecomposition in the hydrogel formulation decreased from 15.4 % for the preparation containing non-encapsulated polyphenol to 9.4 % for the gel containing microencapsulated *trans*-resveratrol, the observed difference being statistically significant. The *trans*-resveratrol photodegradation in the gel was lower than in the emulsion (Fig. 3). This effect can be probably ascribed to limited light-induced

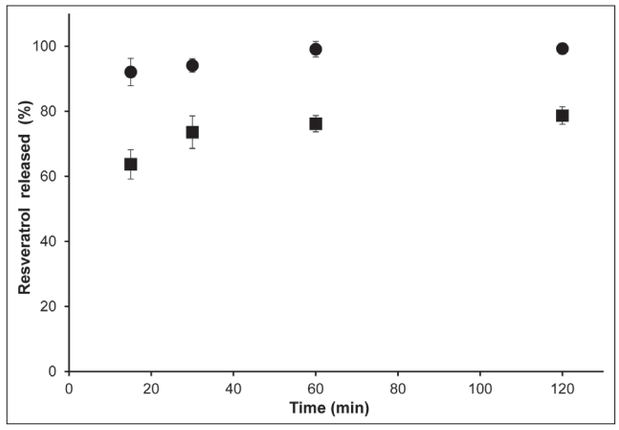

Fig. 2: *Trans*-resveratrol dissolution (filled circles) and release profiles (filled square) from LMs. Values are means ± SD (n= 6).

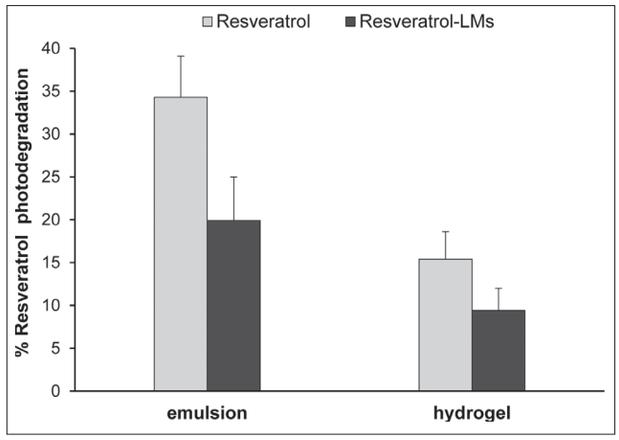

Fig. 3: *Trans*-resveratrol photodegradation (%) in its formulations after 1 h irradiation with the solar simulator. Values are means ± SD (n=6).

reactions between *trans*-resveratrol and the gel vehicle, due to the smaller number of excipients compared to the emulsion formulation (Experimental section). In order to evaluate whether the enhancement of *trans*-resveratrol photostability achieved by the LM system varied with time, additional photolysis experiments were performed after storage of the cream and gel samples at room temperature and in the dark, for 3 months. In the cream, the polyphenol degradation was 38.0±5.2 % for the non-encapsulated resveratrol and 23.1±5.0 % for the microparticle-entrapped polyphenol. The percentage *trans*-resveratrol loss in the gels were 17.5±2.8 % and 10.8±2.3 %, for the free and microencapsulated polyphenol, respectively. Therefore, the photostabilization properties of the LMs were retained after the above time interval.

2.3. In vitro antioxidant activity

The *in vitro* antioxidant activity of *trans*-resveratrol in the cream preparations was measured using the 1,1,-diphenyl-2-picrylhydrazyl (DPPH) assay (Fukumoto and Mazza, 2000). In this methodology, the free radical scavenging property of the polyphenol was evaluated in terms of its potential to reduce the concentration of the stable DPPH free radical. The DPPH radical exhibits a strong absorption at 517 nm which decreases when it is paired by electron or hydrogen donation from antioxidant molecules. The tested creams with *trans*-resveratrol free or encapsulated in the LMs were subjected to the DPPH antioxidant assay. No significant differences were observed by comparing the DPPH radical scavenging activity of the creams and a methanolic solution containing an

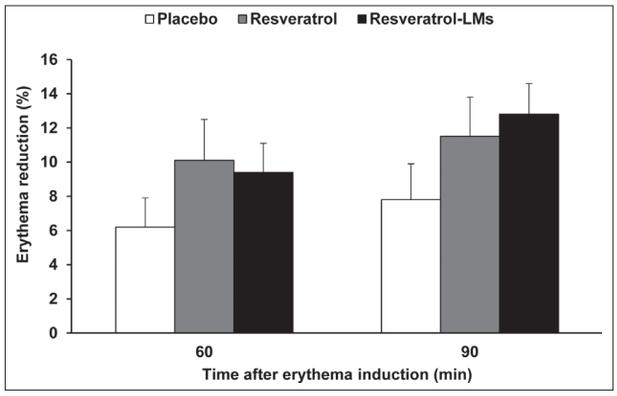

Fig. 4: Effect of topical application of a placebo formulation and creams containing free or microencapsulated *trans*-resveratrol on methyl nicotinate-induced erythema. The preparations were applied to the volar forearm of human volunteers (n=8) and the resulting erythema measured by diffuse reflectance spectroscopy. Values represent means ±SD.

equivalent polyphenol concentration. This result rules out interferences from the formulation excipients. The extent of reduction of DPPH free radicals was 42.3±3.1 % and 44.5±4.4 % (P>0.05) for the preparation containing non-encapsulated or microencapsulated *trans*-resveratrol, respectively. This indicated that the antioxidant activity of the two creams was not significantly different.

2.4. In vivo anti-inflammatory effect

The *in vivo* biological activity of *trans*-resveratrol in the cream preparations was assessed by evaluating its anti-inflammatory effect on methyl nicotinate induced erythema in the volar forearm of human volunteers (Jumbelic and Southall 2006). Diffuse reflectance spectroscopy was employed to measure differences in cutaneous inflammatory responses between the control (placebo) and the sites treated with the creams containing resveratrol free or microencapsulated. When chemical erythema was induced using methyl nicotinate, maximum response was found to occur at 5 min after application and the affected areas returned to baseline 180 min after induction. Based on these data, erythema was measured 60 and 90 min after methyl nicotinate insult. Treatment with either *trans*-resveratrol creams resulted in a significant reduction in erythema response compared with the placebo (Fig. 4). However, there was no significant difference between the erythema mitigation effects attained by the cream formulations based on non-encapsulated or microencapsulated *trans*-resveratrol (Fig. 4).

3. Discussion

The therapeutic potential of *trans*-resveratrol following topical application is hampered by its photoinstability (Montsko et al. 2008; Shi et al. 2008; Sapino et al. 2009; Bonda et al. 2011; Detoni et al. 2012), which reduces its protective activity against skin damage under light exposure. In order to overcome this drawback, several carriers have been proposed including cyclodextrins, yeast cells, liposomes and lipid nanoparticles (Shi et al. 2008; Allan et al. 2009; Sapino et al. 2009; Carlotti et al. 2012; Detoni et al. 2012). The effect of these systems has been evaluated in solutions, suspensions or in topical formulations containing very low (0.005%) resveratrol concentrations (Shi et al. 2008; Allan et al. 2009; Sapino et al. 2009; Carlotti et al. 2012; Detoni et al. 2012). Although these studies provided information on the photostabilization activity of the examined systems, their relevance to real conditions of use of topical preparations is limited. In particular, solutions (Shi et al. 2008; Allan et al. 2009) or suspensions (Carlotti et al. 2012; Detoni et al. 2012) are not suitable for the application of resveratrol preparations to the skin and due to the influence of the medium on the photochemical behaviour, the results reported for the above vehicles (Shi et al. 2008; Allan et al. 2009; Carlotti et al. 2012; Detoni et al. 2012) could be different from those observed in the common skin-care formulations (Block et al 2000). Moreover, evaluation of the photoprotective effect of liposomes and lipid nanoparticles in emulsions and gels has been performed at resveratrol concentrations much lower (Sapino et al. 2009; Carlotti et al. 2012) than those which have been shown to elicit an antioxidant activity in the skin (Ndiye et al. 2011). This is a disadvantage for the applicability of these delivery systems. In addition it should be emphasized that because of its poor percutanous penetration (Scalia et al. 2015), only a small fraction of the topically applied resveratrol reaches the underlying skin layers, where it exerts activity (Scalia et al. 2015). In order to overcome this drawback, in the present study photostability experiments were performed in cream and gel vehicles, representative of typical topical preparations, containing a polyphenol concentration (1%) comparable to the levels which have been reported to achieve *in vivo* a pharmacological effect on the skin (Ndiye et al. 2011). In addition, at variance with previously published studies (Shi et al. 2008; Sapino et al. 2009; Carlotti et al. 2012; Detoni et al. 2012) using irradiation sources (e.g., UVA lamps) that do not simulate the solar UV radiation, in the present investigation sample irradiation was carried out under conditions that mimic natural exposure to sunlight. The use of LMs as carrier for resveratrol, as described here, offers several advantages compared to the previously reported systems based on cyclodextrins, liposomes and lipid nanoparticles, such as simpler preparation method and higher stability. In addition, due to the relatively high loading level of the developed LMs, the required *trans*-resveratrol concentrations could be achieved by introducing less than 5 % (w/w) microparticles in the formulations. This is an advantage, since the spreadability and feeling performance of the topical preparations were not affected.

The results obtained in this study indicated that the incorporation of *trans*-resveratrol in the LM matrix, markedly reduced its photodecomposition respectively by 42 % and 39 %, for the cream and gel vehicles. The protective effect achieved by the developed LMs against resveratrol degradation induced by solar UV radiation was superior to that previously reported for cyclodextrins and lipid nanoparticles in gel and emulsion preparations (Sapino et al. 2009; Carlotti et al. 2012). However, the decomposition of resveratrol following irradiation was not completely inhibited by incorporation in the LMs (Fig. 3). This effect could be traced to the presence of the polyphenol also on the particle surface, as suggested by the release studies, which reduces the resveratrol fraction incorporated inside the lipid particle matrix and hence protected by it.

In order to assess whether the microencapsulation process influenced the functional activity of *trans*-resveratrol, the *in vitro* antioxidant activity of the cream formulations containing free or microencapsulated resveratrol was measured using the DPPH assay. This assay has been widely accepted as a tool for estimating the free radical scavenging activities of antioxidant (Li et al. 2012). Moreover, such methodology has been previously employed to assess the antioxidant capacity of semisolid formulations (Georgetti et al. 2006; Scalia et al. 2013). The obtained data demonstrated that the incorporation of *trans*-resveratrol into the LMs did not influence the antioxidant properties of the polyphenol in the cream preparation.

In addition, the *in vivo* anti-inflammatory activity of *trans*-resveratrol was evaluated by measuring the response to a challenge of a topical methyl nicotinate solution, inducing the development of erythema (vasodilatory response) in the volar forearm of human volunteers. Several methods have been reported to measure superficial vasodilatation, however the non-invasive Diffuse Reflectance Spectroscopy was selected for this study, because of its higher sensitivity (Andersen and Bjerring 1990). The results obtained from this *in vivo* assay (Fig. 4) indicated that the encapsulation of resveratrol in the LMs did not alter its anti-inflammatory activity in the cream formulation. To the best of our knowledge, this is one of the first example of an in vivo evaluation of the activity of encapsulated resveratrol.

In conclusion, the data described in the present study indicated that encapsulation of *trans*-resveratrol in LMs represents an effective strategy for decreasing the photolability of the polyphenol, without affecting its biological properties. The reduction in the loss of *trans*-resveratrol induced by light exposure should ensure enhance activity of the polyphenol topical preparation during usage.

4. Experimental

4.1. Materials

Trans-resveratrol was supplied by Fagron Italia (Bologna, Italy). Hydrogenated phosphatidylcholine was a gift from Cargill (Hamburg, Germany). Tristearin, methyl nicotinate, 1,1,-diphenyl-2-picrylhydrazyl (DPPH), high-performance liquid chromatographic (HPLC)-grade methanol and water were from Sigma-Aldrich (Steinheim, Germany). The excipients for the cream and gel preparations were obtained from Croda (Snaith, UK), Seppic (Paris, France) and Fagron Italia (Bologna, Italy). All other reagents and solvents were of analytical grade from Sigma.

4.2. High-performance liquid chromatography

The HPLC system comprised a Model LabFlow 3000 pump (LabService Analytica, Bologna, Italy), a Model 7125 injection valve with a 20 µl sample loop (Rheodyne, Cotati, CA, USA) and a Model 975-UV variable wavelength UV-vis detector (Jasco, Tokyo, Japan) set at 306 nm. Data acquisition and processing were performed with a personal computer using Borwin software (JBMS Developpements, Le Fontanil, France). Sample injections were performed with Model 80365 syringe (10 µl; Hamilton, Bonaduz, Switzerland). Separations were achieved on a 5-µm Zorbax SB-C18 column (150 mm x 4.6 mm i.d.; Agilent Technologies, Waldbronn, Germany) fitted with a guard column (5-µm particles, 4 mm x 2 mm i.d.; Phenomenex, Torrance, CA, USA) and eluted isocratically, at a flow-rate of 0.8 ml/min, with methanol-water (65:35, v/v) containing 0.4% (v/v) acetic acid. Chromatography was performed at ambient temperature. The identity of *trans*-resveratrol peak was assigned by co-chromatography with the authentic standard. Quantification was carried out by integration of the peak areas using the external standardization method. Calibration curves were linear over the range 2.0- 30.0 µg/ml, with correlation coefficients greater than 0.998. The precision of the method, evaluated by repeated analyses (n=6) of the same sample solution containing resveratrol at levels of 2.0 and 30.0 µg/ml, was demonstrated by relative standard deviation values lower than 5.8 %.

4.3. Microparticle preparation

LMs were prepared by adding hot (75 °C) deionized water (40 ml) containing the surfactant (0.7 %, w/v) to the molten lipid phase (3.8 g of tristearin), in which *trans*-resveratrol (0.7 g) was dispersed. The mixture was then subjected to high-shear mixing (17500 rpm for 1.5 min) using an Ultra-Turrax T25 mixer (IKA-Werk, Staufen, Germany) at 75 °C. The resulting oil-in-water emulsion was rapidly cooled at room temperature under magnetic stirring and the formed suspension was subjected to centrifugation (6000 rpm for 15 min) and lyophilization to obtain water-free microparticles.
Unloaded particles were also prepared with the same procedures, by omitting resveratrol.

4.4. Microparticle characterization

Microparticle morphological structure was observed by optical microscopy (B-500 TPL microscope, Optika Microscopes, Bergamo, Italy).
The particle dimension was evaluated by computerized image analysis (Micrometrics™ camera 122CU and software vision 2.02) on a minimum of 150 particles using the B-500 TPL optical microscope.
The amount of *trans*-resveratrol entrapped in the LMs was determined by dissolving the microparticles (10 mg) in ethanol (5 ml) under heating (75 °C for 5 min) and sonication (10 min), in sealed glass vials. The obtained sample was diluted to volume (20 ml) with methanol, filtered (0.45 µm membrane filters) and assayed by HPLC. The encapsulation efficiency was calculated as the percentage ratio between the quantity of resveratrol entrapped in the microparticles and the amount of polyphenol initially added to the melted lipid phase. The results were the average of at least three determinations.
Trans-resveratrol dissolution and release were assessed by adding previously sieved (63 µm) resveratrol (ca. 0.8 mg) or LMs, containing an equivalent amount of the polyphenol, to 100 ml of phosphate buffer (0.05 M, pH 7.4), containing polysorbate 20 (0.5%, w/w) as solubilizer to ensure sink conditions (Scalia et al. 2015). The samples were kept under mechanical stirring at 50 rpm and 32 °C. At appropriate time intervals, 1-ml aliquots of the medium were withdrawn and replaced with an equal volume of fresh fluid. The test samples were filtered (0.45 µm) and assayed for resveratrol by HPLC, as outlined above. The polyphenol release (%) was calculated from the total *trans*-resveratrol content of each LM preparation. This was determined by extraction of the particles, after the release experiment, using the method described above. A minimum of six replicates were performed for each formulation.

4.5. Formulations

Photolysis experiments were performed on oil-in-water (o/w) emulsion and hydrogel preparations, containing non-encapsulated *trans*-resveratrol (1.0%, w/w) in conjunction with blank LMs or an equivalent amount of polyphenol loaded in LMs.
Hydrogels were prepared by dispersing under mechanical stirring (70 rpm, RW-20, IKA-Werk) hydroxyethyl cellulose (2%, w/w) in hot (60 °C) deionized water containing sodium methylparaben (0.25%), sodium propylparaben (0.15%), benzyl alcohol (0.5%) and EDTA (0.1%). *Trans*-resveratrol (solubilized in ethanol), unloaded or loaded LMs (dispersed in water) were then added to the formulation at a temperature of about 35 °C , under gentle stirring.
The emulsion excipients were: cetearyl isononanoate (7.5%), glyceryl stearate (0.5%), Phenonip® (0.8%; phenoxyethanol and parabens), benzyl alcohol (0.5%) and Montanov™ 82 (5.0%; cetearyl alcohol and coco-glucoside) for the internal phase and glycerin (2.0%), EDTA (0.1%), ethanol (3.0%) and deionized water (qs 100%) for the external phase. The creams were prepared by separately heating the oil- and aqueous-soluble components at about 70 °C and then adding the aqueous phase to the oil phase while mixing with an Ultra-Turrax T-18 at 7000 rpm for 2 min. *Trans*-resveratrol (solubilized in ethanol), unloaded or loaded LMs (dispersed in water) were added in the cooling phase of the emulsion preparation at about 35 °C, under gentle stirring.

4.6. Photodegradation studies

Aliquots (ca. 40 mg) of the test emulsion or hydrogel preparations containing free or microencapsulated *trans*-resveratrol were evenly spread by means of a syringe onto the bottom of beakers (surface area 12.5 cm^2) and irradiated with a solar simulator (Suntest CPS+, Atlas, Linsengericht, Germany) for 1 h. The solar simulator emission was maintained at 500 W/m^2, corresponding to an UV irradiance of 54.9 W/m^2, comparable with natural sunlight (Dondi et al. 2006). After the exposure interval, the content of the beaker was quantitatively transferred into a 20-ml calibrated flask with ethanol (2x5 ml) and subjected to heating (70 °C) and sonication (10 min). The obtained sample was diluted to volume (20 ml) with methanol, filtered (0.45 µm membrane filters) and analysed by HPLC. The degree of photodegradation was evaluated by measuring the percentage of recovered *trans*-resveratrol with respect to the non-irradiated sample. The results were the average of at least six experiments.

4.7. In vitro antioxidant activity

The *in vitro* assay of the antioxidant activity of the cream preparations containing free or microencapsulated *trans*-resveratrol (1.0%) was performed on a portion of the methanolic solution obtained by extraction of the examined creams with methanol (2 x 10 ml). The antioxidant activity was measured by the DPPH assay, according to the method of Fukumoto and Mazza (2000) with minor modifications. Briefly, aliquots (0.5 ml) of the test samples from the studied formulations, were added to 1.5 ml of the DPPH stock solution (0.1 mM in methanol). The mixture was stirred vigourosly and incubated for 30 min in the dark at room temperature. Then the sample absorbance was measured at 517 nm (Uvikon 923 spectrophotometer, Kontron Instrument, Zurich, Switzerland). The control solution contained the same DPPH concentration in methanol. The DPPH radical scavenging activity of the *trans*-resveratrol formulations was calculated according to the following equation:

$$\text{scavenging (antioxidant) activity (\% inhibition)} = \frac{\text{Control absorbance - Sample absorbance}}{\text{Control absorbance}} \times 100$$

Samples were tested in sextuplicate.

4.8. In vivo evaluation of anti-inflammatory effect

The *in vivo* assay was carried out on eight volunteers of both sexes, aged 23-40 under the supervision of a dermatologist. The study protocol was approved by the local Ethics Committee (Comitato Etico della Provincia di Ferrara) and complied with the Declaration of Helsinki guidelines. The volunteers underwent a careful dermatological visit, received detailed information about the study and written informed consent was collected prior to any experiment. Exclusion criteria were any sign of photosensitivity, urticaria, allergies, infection or inflammation of the skin, involvement in similar clinical test in parallel or within the preceding three months. All experiments were performed in the same room under controlled ambient conditions (temperature, 23 °C and relative humidity, 50%) and each volunteer was acclimatized for 15 min before testing.
Three delineated areas (diameter, 1.5 cm) on the volar forearm of the volunteers were treated (5 min) for erythema induction with an aqueous solution of methyl nicotinate (36.5 mM, 50 ml) (Jumbelic and Southall 2006) which was distributed on a blotting paper disk of 1.8 cm^2 (28,4 ml/cm^2). After 5 min treatment, the creams (0.1 ml) containing free or microencapsulated *trans*-resveratrol were applied to two of the delineated areas, leaving the third area for the placebo formulation. After an application time of 10 min, the remaining preparation was removed from the treated areas and at prefixed time points, the Erythema Index (EI) was measured using the Dermaspectrophotometer, DSM II Color Meter (Cortex Technology, Hadsund, Denmark). This apparatus consists of a handheld color measuring system providing both erythema and melanin index, as well as color measurements. The Dermaspectrophotometer emits light at two defined wavelengths (586 nm and 655 nm) and photodectors measure the light reflected by the skin. The instrument probe was placed gently on the skin and the results, expressed as Erythema Index, were immediately displayed. Measurements were also performed before the induction of erythema, to establish the baseline level.

4.9. Statistical analysis

Data were analysed using Student's t-test, analysis of variance (ANOVA) and Tukey's post-test. Differences were considered significant for P values < 0.05. Statistical analysis was carried out using GraphPad Instat software (Graphpad, San Diego, CA).

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Conflicts of interest: None declared.

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