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The Topical Protective Effect of Soybean-Germ Oil against UVB-Induced Cutaneous Erythema: an *in vivo* Evaluation

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The preparation and detailed composition of an oil newly extracted from pure soy germ (not less than 96% hypocotyle) are presented. Experiments *in vivo* showed that soybean-germ oil (SGO) possesses a remarkable protective activity against UVB-induced skin inflammation, exceeding that of tocopherol acetate by a factor of 2. These results suggest that SGO might have interesting therapeutic and cosmetic applications in the management of skin diseases initiated, sustained, or exacerbated by an overproduction of free radicals.

Keywords: Soybean-germ oil; Analysis; Erythema; Tocopherol acetate; Antioxidant

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Introduction

The skin is exposed to a broad variety of biological, chemical, and physical attacks. Ultraviolet light, the most thoroughly studied agent in the last class, is known to cause damage resulting in both precancerous and cancerous skin lesions, besides a general acceleration of skin ageing [1]. In fact, UVB and UVA (the biologically relevant portions of solar UV that reach down to the earth surface) have been shown to penetrate epidermal and dermal layers, where they can provoke chemical changes and degradation of cellular components (lipids, proteins, and DNA) and extracellular elements (collagen and elastic fibers).

Reactive oxygen species (ROS) are believed to be largely responsible for some of these deleterious effects [2]. In particular, prolonged exposure of the skin to UV results in a marked decrease of its antioxidant content [3]. Overproduction of nitric oxide by keratinocytes seems to have a major role in the integrated response leading to erythema production and other inflammatory events following UV exposure [4, 5]. The endogenous antioxidant capacity of the skin is a major determinant of its response to oxidative stress. In fact, topical administration of antioxidants has recently proven effective in protecting the skin against UV-mediated oxidative damage [2, 6−9], and provides the most straightforward way to strengthen the endogenous protection system.

Soybean-germ oil (SGO) deserves an important place among nutraceuticals [10] because of its very high content in tocopherols, phytosterols, polyunsaturated fatty acids (PUFAs, both omega 3 and omega 6), carotenoids, and other vitamins [10, 11]. Moreover, SGO cholesterol-lowering activity was substantiated in rats [11, 12] as well as in human subjects [13, 14]; the effect was attributed to phytosterols, known to inhibit the absorption of cholesterol and to increase its fecal elimination [14]. However, all relevant scientific work published to date has been carried out on oils extracted from partially enriched soy germ, owing to inherent limitations in the current separation techniques. We overcame this drawback by adapting rice milling technology to the soybean refining process, so achieving a rapid and efficient separation of soybean bran and cotyledon from hypocotyle. An analysis of our SGO, which is a hexane extract of pure (no less than 96%) soy hypocotyle, is presented here with the results of Rancimat tests, demonstrating its very good stability to thermal oxidation in spite of its very high content of PUFAs.

The specific aim of the present work was to evaluate *in vivo* the ability of SGO to protect human skin from UVB-induced erythema. This inflammatory response that can be easily quantified by means of reflectance spectrophotometry is regarded as one of the most suitable indicators for evaluating *in vivo* skin damage after acute UV exposure and provides a useful tool to assess the radical-scavenger activity of topically applied compounds [2, 6, 15].

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Results and discussion

We developed an improved industrial technique to completely separate soybean bran and cotyledon from the hypocotyle that makes up about 3% in weight of the whole seed. This objective was achieved by adapting the rice milling technique to the soybean that was actually milled rather than broken as in customary processing. The resulting soybean germ contained no less than 96% hypocotyle, as determined by microscopy. The hexane extract of such a preparation from non-GMO (genetically modified soy) Italian cultivars afforded the SGO used in the present work. Japanese researchers had previously compared the composition of a partially enriched SGO to that of soybean oil [11]. Using official methods for oil analysis [16] we did the same for our SGO. Its main fatty acid components were linoleic, linolenic, and oleic acid (Table 1).

It was very rich in tocopherols (total content 4.30 g/kg) while only traces of tocotrienols were present (Table 2).

Although isoflavones are particularly abundant in soybean germ (total content about 18 g/kg), only 7–8 mg/kg were present in our SGO (glycitein derivatives 3 mg/kg, daidzein derivatives, and genistein derivatives 2 mg/kg each). The total sterol content was 45 g/kg, most abundant being beta-sitosterol (57.8%), Δ-7-stigmastenol (20.5%), Δ-7-avenasterol (6.9%), stigmasterol (6.3%), and campesterol (5.6%). The antioxidative properties of SGO were evaluated by adding a modest percentage of it (5–15%) to fish oil (Anchovy) and measuring the resistance of the mixture to autooxidation by Rancimat-test [17] by comparison with pure fish oil and fish oil to which tocopherol acetate had been added (5000 ppm). The oils were heated to 110°C and a steam of air was bubbled through them. Ensuing oxidation generates volatile substances that are carried by the air stream to a collector containing bidistilled water, whose conductance increases. The induction time is taken as a measure of the resistance to oxidation of the oil under study (Table 3). SGO showed an outstanding power to protect other highly unsaturated oils when added to them in percentages as low as 10%.

Table 1. Fatty acids profile of SGO (weight %).

Fatty acid†	%	Fatty acid	%
C14:0 myristic	0.2	9*t*- C18:1 elaidic	0.06
C16:0 palmitic	12.7	C18:2 linoleic	56.5
C16:1 palmitoleic	0.1	C18:3 linolenic	15.6
C17:0 heptadecanoic	0.1	C18:2 + C18:3‡	0.2
C17:1 heptadecenoic	0.1	C20:0 arachidic	0.4
C18:0 stearic	3.6	C20:1 eicosenoic	0.1
C18:1 oleic	10.6	C22:0 behenic	0.2

† Free acidity as oleic acid: 1.9%.
‡ Not defined stereoisomers.

Table 2. Tocopherols and tocotrienols content of SGO.

Tocopherol	mg/kg
α-tocopherol	1967
β + γ-tocopherol	2156
δ-tocopherol	237
Total tocopherols	4360
Total tocotrienols	3

Table 3. Rancimat tests.

Sample	Time (h 10^{-2})
Fish oil	0.16
Fish oil + Tocopherol acetate (5000 ppm)	1.06
Fish oil + 5% SGO	1.99
Fish oil + 10% SGO	2.16
Fish oil + 15% SGO	2.28

To assess the protective effect of SGO against UV-B-induced erythema, the degree of skin reddening was monitored in human volunteers by means of reflectance spectrophotometry. Before discussing the results, a brief methodological comment is in order. From reflectance spectra, generally in the range of 400–700 nm, the values of different colour space systems (CIELab, Lch, etc.) can be obtained using different CIE illuminants (C, D_{65}, D_{50}, A, etc.) and 2 or 10° illuminant observer. From spectral data it is possible to calculate at different wavelengths the relative reflectance or the logarithm of inverse reflectance (LIR), which is related to the light absorption of skin chromophores (hemoglobin, melanin, etc.). The erythema index (EI) obtained from skin reflectance spectral values is thought to give a more accurate and reliable evaluation of skin erythema [18]. Since this is due to an increased hemoglobin content in skin vessels, EI values are calculated by subtracting LIR values at 510 and 610 nm (mainly related to melanin absorbance) from the sum of LIR values at 540, 560, and 580 nm, the absorption peak wavelengths for hemoglobin [15, 19].

The time course of erythema for skin patches treated with SGO after UVB irradiation is shown in Figure 1. From Δ.E.I. *vs* time plots, areas underlying response-time curves (AUC_{0-72}) were calculated and are reported in Table 4. By comparison with controls, it is apparent that SGO exerted on the skin a significant protection against UVB-induced erythema. In this regard it proved more active (percentage inhibition erythema–PIE 46.8%) than tocopherol acetate (TOC) (PIE 21.5%).

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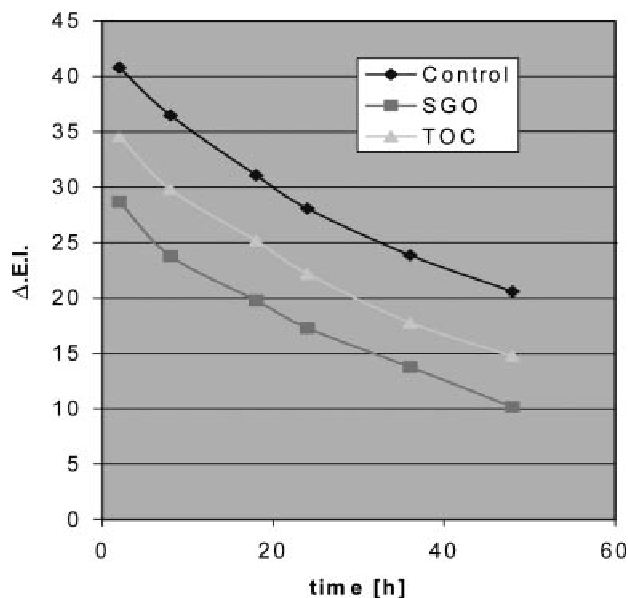

Figure 1. Typical erythema index (Δ.E.I.) *vs* time plots for one subject. SGO was applied to skin patches immediately after exposure to UVB and left there for 3 h before reflectance measurements.

Table 4. AUC_{0-48} values obtained by treating skin patches immediately after exposure to UVB, with either TOC or SGO.

Subject	Control	TOC	SGO
A	1328.6	1046.0	817.4
B	1475.2	1121.7	702.8
C	1298.3	1078.5	595.3
D	1358.5	1121.4	808.3
E	1246.8	984.3	748.7
F	1427.4	1034.8	652.5
Average S.D.	1355.8 ± 84.0	1064.4 ± 53.6	720.8 ± 87.7
P.I.E.	–	21.5	46.8

Conclusion

The present *in vivo* findings show that SGO possesses a remarkable protective activity against UVB-induced skin inflammation, probably due to its radical-scavenging components, mainly tocopherols and polyunsaturated fatty acids. These results suggest that SGO could have important therapeutic and cosmetic applications in the management of skin diseases initiated, sustained, or exacerbated by an overproduction of free radicals. The peculiar composition of its large unsaponifiable fraction confers on SGO an excellent resistance to thermal oxidation. Even more remarkably, as measured by the Rancimat test, was the outstanding power to protect from it other highly unsaturated oils when added to them in percentages as low as 10%. This property obviously recommends SGO as a stabilizing additive for fatty foods, cosmetics, and nutraceuticals.

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Experimental

Analysis

SGO was produced in collaboration with Italgreen-oil, a division of Casa Olearia (Verona, Italy). Analytical TLC was carried out using plates Alufolien Kieselgel 60F 254 (Merck, Darmstadt, Germany). Low-resolution mass spectra (LRMS): Finnigan-MAT TSQ70 (Thermo Electron Corporation, Bremen, Germany) in chemical ionization with isobutane as reactant gas. GC analyses were carried out either on a Shimadzu GC-14 B with FID detector (Shimadzu, Duisburg, Germany) or an Agilent 6890 with Mass detector Agilent 5973 network (Agilent, Palo Alto, CA, USA). HPLC analyses were carried out on an Amersham Akta Purifier system (Amersham Biosciences, Uppsala, Sweden) equipped with a UV-Vis triple wavelength detector and on-line monitors for pH, conductibility, and temperature. Fatty acid profile: GC analysis of methyl esters (NGD C 42-76; EC reg. 2568/91 and sub. Amend., Ann. XA-XB). Phytosterols profile: GC analysis (NGD C 71-89, C 72-89; EC reg. 2568/91 and sub. Amend., Ann. V). Tocopherols and tocotrienols: HPLC determination (NGD C 69-85; ISTISAN report 96/34). Isoflavones: analytical protocol based on a published method [20].

Rancimat test

The standard Rancimat test was carried out using a Model 679 Rancimat apparatus (Methrohm Co., Basel, Switzerland). 3.5 g of oil were weighed in the reaction vessel that was placed in the heating block kept at 110°C. Air flow was set at 20 L/h for all determinations. Volatile compounds released during the degradation process were collected in a receiving flask filled with 60 mL distilled water. The conductivity of this solution was measured and recorded. All determinations were carried out in duplicate. Results are given as induction times, expressed in $h \cdot 10^{-2}$ (Table 3).

In vivo evaluation of the photoprotective effect

UVB-induced skin erythema was monitored with a reflectance visible spectrophotometer X-Rite mod. 968, having 0° illumination and 45° viewing angle. The instrument was calibrated with a supplied white standard traceable to the National Bureau of Standard's perfect white diffuser. It was connected to a personal computer which performed all color calculations from the spectral data by means of the Spectrostart program supplied with the instrument. Reflectance spectra were obtained over the wavelength range 400–700 nm using illuminant C and 2° standard observer.

In vivo experiments were performed on 6 healthy volunteers of both sexes with skin types II and III and a mean age of 31 ± 9 years. Volunteers, after receiving full information on the nature of this study and the procedures involved, gave a written consent to them. Subsequently, they did not report any discomfort or other adverse reaction. The subjects, who were not on medication at the time of the study, were rested for 15 min prior to the experiments. Room conditions were set at 22 ± 2°C and 40–50% relative humidity.

Skin erythema was induced using an ultraviolet lamp mod. UVM-57 (UVP, San Gabriel, CA, USA), which emitted in the range

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290−320 nm with an output peak at 302 nm. The flux rate measured at the skin surface was 0.80 mW/cm^2. For each subject the minimal erythema-inducing dose (MED) was determined preliminarily and an irradiation dose twice the MED was then used throughout. For each subject, six circular areas (1 cm^2) to be exposed to UVB irradiation were demarcated on the ventral surface of one forearm using a template and permanent ink. Immediately after exposure, 200 µL of SGO were applied to these patches for three hours using a Hill Top chamber (Hill Top Research Inc., Cincinnati, OH, USA). Two control patches were exposed but left untreated. Finally the chambers were removed, the skin surfaces gently washed with water to remove the solutions and dried. The induced erythema was monitored for 48 h as described above.

From the skin spectral data following UVB exposure the erythema index (EI) was calculated using the following equation, where 1/R are inverse reflectances at a specific wavelengths (560, 540, 580, 510, 610 nm) [15, 19]:

$$EI = 100 \left[\text{Log}\frac{1}{R_{560}} + 1.5\left(\text{Log}\frac{1}{R_{540}} + \text{Log}\frac{1}{R_{580}}\right) - 2\left(\text{Log}\frac{1}{R_{510}} + \text{Log}\frac{1}{R_{610}}\right)\right]$$

EI baseline values taken before UVB irradiation were subtracted from post-irradiation EI values to give corrected erythema index values (ΔEI). For each patch, the area (AUC) underlying the ΔEI-time curve was computed using a suitable software.

AUC values were taken to be inversely related to the ability of tested substances to inhibit skin erythema. To better compare the efficacy of different products, the percentage inhibition of UVB skin erythema (PIE) was calculated from AUC values using the following equation, where $AUC_{(C)}$ was the area for controls patches and $AUC_{(T)}$ the areas for patches treated with products to be tested. Statistical analysis was performed by using Student's *t*-test.

$$\text{Inhibition \% (PIE)} = \frac{AUC_{(C)} - AUC_{(T)}}{AUC_{(C)}} \times 100$$

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