The Active Ingredients Mixture of Olives Provides Skin Whitening and Age Spot Reduction

Author: Maria Lueder, Qenax AG, Switzerland

Keywords: Skin Whitening, Age Spot Reduction, Olea Europaea, Hydroxytyrosol, Oleuropein, Pheomelanin, Eumelanin, Glutathione

Abstract

The olive tree, *Olea europaea*, is native to the Mediterranean basin and parts of Asia Minor. Native olive oil has often been used in cosmetic formulations but other interesting ingredients from the fruit and the leaves for a long time were not investigated for use in cosmetic products. The fruit and the leaves contain high amounts of polyphenols. The major polyphenol is Oleuropein. With increasing maturity of the olive hydroxytyrosol will be released from the Oleuropein, which is even stronger in anti-oxidative power than the Oleuropein.

A standardised olive extract (Tradename: Cayoma® Olive) containing high amounts of polyphenols and hydroxytyrosol has been prepared to evaluate skin whitening and age spot reduction effects. The cosmetic active was tested both *in vitro* and *in vivo*.

Melanin reduction as a direct measure for skin lightening properties and age-spot reduction was measured *in vitro* on primary human melanocytes. Additionally glutathione was also measured *in vitro* on primary human melanocytes. A high glutathione level in melanocytes directs the production of melanin into the soluble and lighter brown pheomelanin versus dark and insoluble eumelanin which supports skin lightening.

The *in vivo* test was designed as a double blind, placebo controlled study on the hands of twelve Caucasian female volunteers aged between 46 and 72. The study duration was three months, with readings before the first application, after four, eight and twelve weeks.

A significant melanin reduction of up to 50% and a significant increase of the glutathione level up to 55% could be measured in vitro. The confirmation *in vivo* showed not only a significant reduction of age spot colour but also a colour reduction of the whole skin area on the hands.

Introduction

Hippocrates used olive leaves for wound healing and Dioskurides applied them in fomentations against skin inflammation, abscess thrush and slowly healing wounds. Today it is known that the high polyphenol content with powerful anti-oxidative properties is responsible for these effects.

Depending on the olive's degree of ripeness, these secondary plant components[1] are found in the cold pressed oil (approx. 0.1%) and develop effects similar to omega-3 fatty acids, especially the Oleuropein. With increasing maturity of the olive, hydroxytyrosol will be released which is stronger in anti-oxidative power (Figure 1). Like resveratrol, hydroxytyrosol is a very strong antioxidant. As hydroxytyrosol is amphiphilic, it can develop its effect as a powerful radical scavenger in lipophilic cell membranes as well as in the hydrophilic cell plasma.

Publications show that hydroxytyrosol protects human melanocytes *in vitro* from protein damage, induced by long-wave UV light, and reduces the release of inflammation inhibitors like Cox-2 in macrophages[2,3].

Glutathione is a peptide, consisting of three amino acids Glutamic acid, Cysteine and Glycine. It is present in almost all cells in higher concentrations and belongs to the most important natural antioxidants of the body. At the same time it is a reservoir for Cysteine. Glutathione protects cellular macromolecules like proteins and membrane lipids against free radicals (reactive oxygen species ROS). An increased level of glutathione stimulates the formation of the lighter and soluble pheomelanin versus the darker, insoluble eumelanin[4]. Tyrosinase, the rate limiting enzyme of melanogenesis, catalyses the hydroxylation of L-tyrosine to DOPA and the oxidation of DOPA to DOPAquinone. If cysteine or glutathione is present, it reacts with DOPAquinone to produce cysteinylDOPA and the benzothiazine derivatives of pheomelanin[5] (Figure 2).

Cayoma® Olive, a standardised extract from olive fruit and olive leaves with a high content of active ingredients has been developed and tested against age spots and for skin lightening *in vitro* and *in vivo*.

Photo of olive branch

The *in vitro* effect of Cayoma® Olive was tested twofold. The melanin reduction and the glutathione increase as result of a treatment with the standardised olive extract were measured on primary human melanocytes. The increase of glutathione was measured to demonstrate the formation towards the lighter and more soluble pheomelanin.

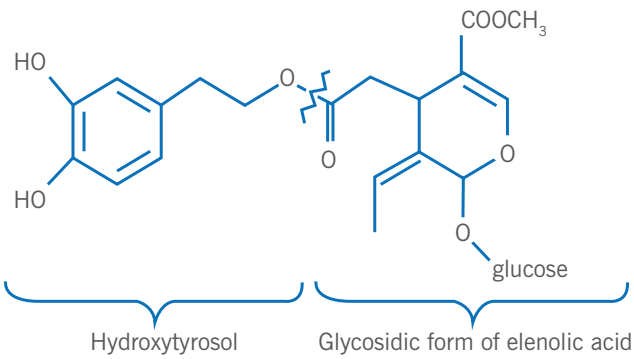


Figure 1. Structure of Oleuropein

Materials and Methods

An active ingredient Cayoma® Olive based on an aqueous alcoholic solution with at least 8% hydroxytyrosol and 12% olive polyphenols was used for the *in vitro* studies. All tests were run at independent test institutes(Note 1).

For the *in vivo* study a hand cream formulation with 0.2% of Cayoma® Olive had been prepared with the following INCI declaration:

Aqua, Dicaprylyl Ether, Glycerin, Butyrospermum Parkii Butter, Squalane, Alcohol, Cera Alba, Prunus Amygdalus Dulcis Oil, Behenyl Alcohol, Caprylic/Capric Triglyceride, Cetearyl Alcohol, Hydrogenated Lecithin, Phenoxyethanol, Sodium Hyaluronate, Xanthan Gum, Ethylhexylglycerin, Hydroxyethylcellulose, Parfum, *Olea Europaea* Leaf Extract, *Olea Europaea* Fruit Extract, Maltodextrin, Ceramide III

Melanin Reduction

Primary human melanocytes (Lifeline Technologies) were grown for 24 hours before the cells were exposed to a Cayoma® Olive concentration in the range of 0 – 0.0078% for a period of 48 hours.

Afterwards the cells were incubated with a 0.1% L-Dopa containing medium. Optical Density (at 405nm) measurements were then taken after 0 min and 140 minutes in four replicate wells.

Melanin content was then calculated by subtracting the values of the blank and at 0 min, and then converted using a standard curve. Results are presented as a percentage of the negative control (untreated cells). Prior cytotoxicity tests to the study with the highest used extract concentration did not show any adverse effects.

Glutathione Increase

The assay is based on the conversion of a luciferin derivative into luciferin in the presence of glutathione. The signal generated in a coupled reaction with firefly luciferase is proportional to the amount of glutathione present in the sample(6).

Primary human melanocytes were grown for 24 hours before the cells were stimulated with Cayoma® Olive for 48 hours, then analysed for glutathione concentration. Prior cytotoxicity tests to the study with the highest used extract concentration did not show any adverse effects.

In vivo Test of Skin Lightening and Age Spot Reduction on Human Volunteers

Female Caucasians, aged between 46 and 72 years were chosen for the study. The aim was to demonstrate the effect of Cayoma® Olive on the reduction of age spots and skin lightening properties in a double blind, placebo controlled study. The study duration was 3 months with measurements before the study, after 4, 8 and 12 weeks. Among the 12 participants, 4 had normal skin, 3 had dry skin, and 5 had very dry skin.

The volunteers had to apply a hand cream with 0.2% of Cayoma® Olive 3 times daily using 0.5 gram of the cream

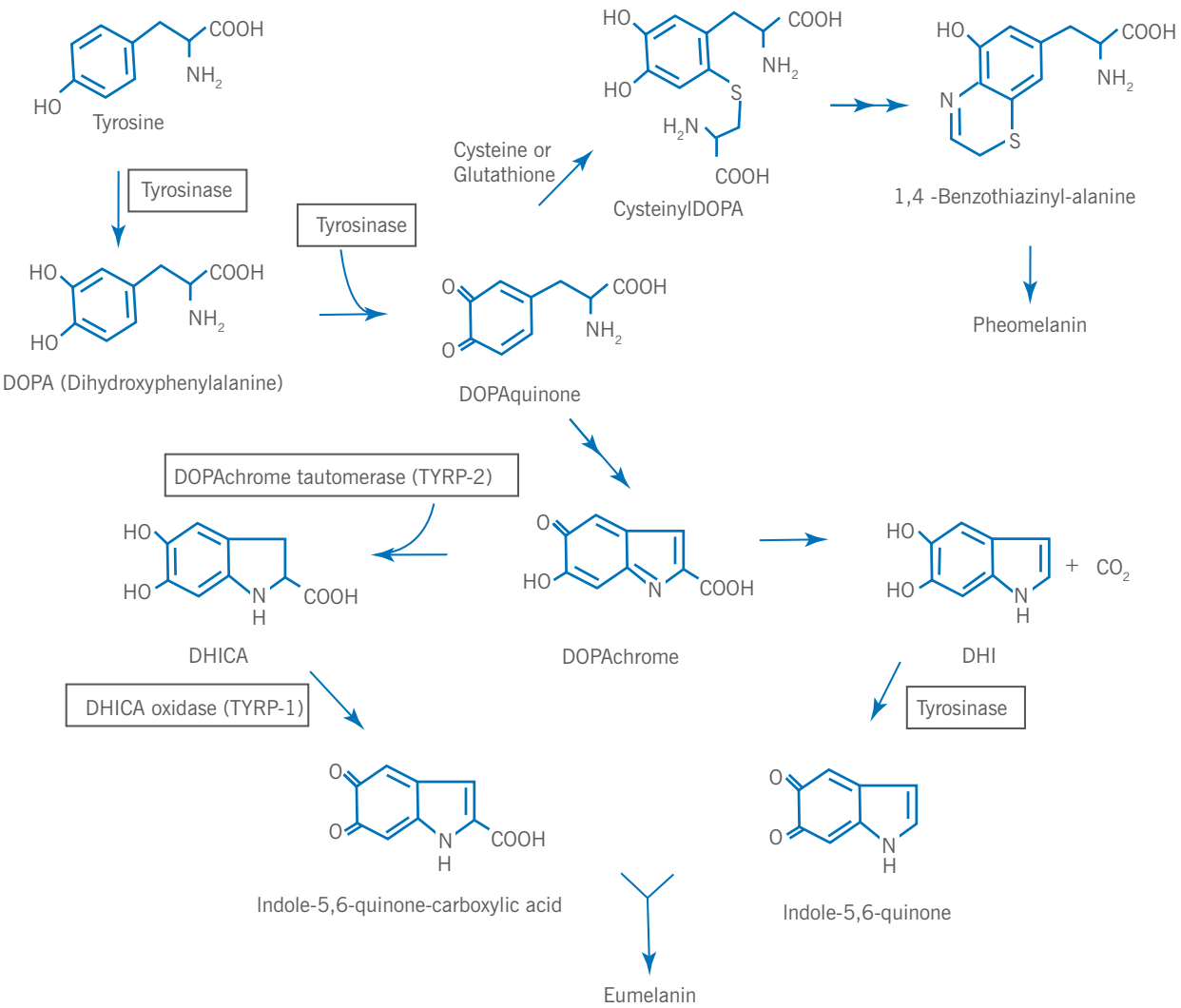


Figure 2. Process of melanogenesis within epidermal melanosomes

per application. One hand was treated with the cream, the other was treated with a placebo cream. During the study the volunteers were asked to avoid direct UV sun light.

The measurements were taken with the Skin Pigmentation Analyzer SPA 99 (Courage & Khazaka, Cologne, Germany). The measuring head has a diameter of 2mm, and spot sizes above 3mm are therefore considered sufficient to avoid false positive results.

In the SPA 99, the probe head emits three specific light wavelengths to the skin surface in a controlled environment. A receiver module measures the light reflected by the skin, and the microprocessor within the device calculates the quantity of light absorbed. The result is displayed on a digital readout as a 0-99 index (Figure 3).

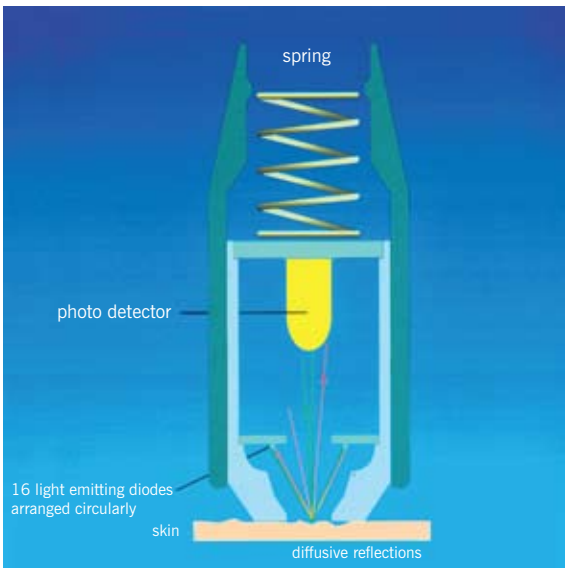


Figure 3. Measuring principle if the SPA 99

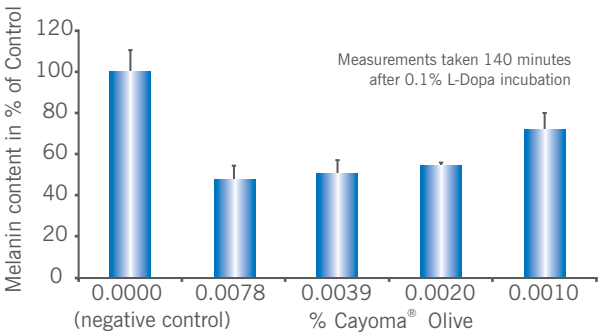
4 defined spots were chosen and measured at every defined date. The area free of spots was taken to measure the overall effect of the cream in direct comparison to the spot area. Additionally photos were taken from the hands of the volunteers prior to the start and at every measurement date of the study. The melanin index of the chosen skin areas was measured. Measurements were performed under controlled environmental conditions (T 22.5±1.5°C; RH 50±10%).

The Melanin Index is derived from the reflectance spectrum, and can be used as the primary measure of skin colour[7,8]. The Melanin Index is a unit-less, continuous variable objectively quantifying skin colour[9].

Results

Melanin Reduction

The highest concentration induced the largest average reduction in melanin content, with values falling to the range of 50-65% of control (Figure 4).



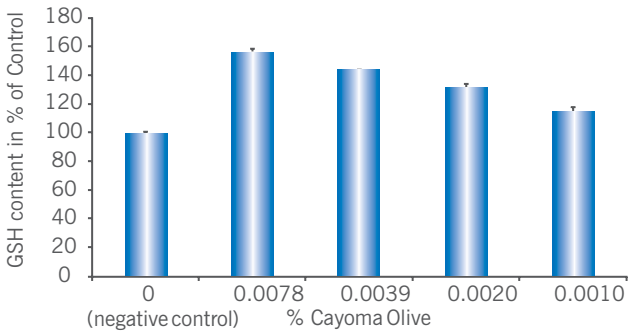
Melanin content after 48h extract exposure
Mean + Standard Deviation

Figure 4. Melanin Reduction at a seeding density of 10'000 cells per well

The analyses conducted show that Cayoma® Olive is able to decrease significantly the melanin content in primary human melanocytes following 140 minutes treatment in the range of 0.002 – 0.008%.

Glutathione Increase

GSH content significantly increased in cells treated with Cayoma® Olive. The effect was dose dependent, with the highest extract concentration (0.0078%) inducing the largest increase in GSH (+55%).



Glutathione Increase after 48h extract exposure
Mean + Standard Deviation

Figure 5. Glutathione Increase at a seeding density of 10,000 cells per well

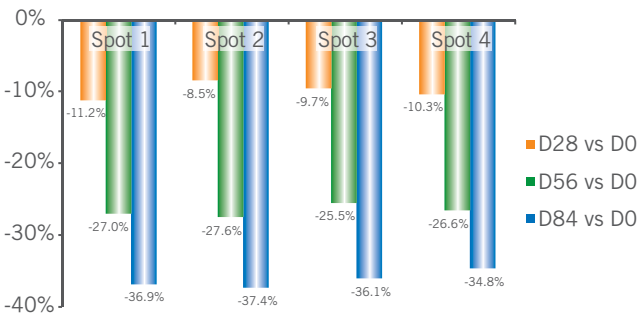
The analyses conducted show that Cayoma® Olive is able to increase significantly the glutathione content in primary human melanocytes following 140 minutes treatment in the range of 0.002 – 0.008% (Figure 5).

In vivo Test of Skin Lightening and Age Spot Reduction on Human Volunteers

The verum showed to be efficient in depigmenting age spots significantly at 8 weeks and 12 weeks, versus the placebo (Figure 6).

The verum showed to be also efficient in depigmenting adjacent spot-free zones (unspotted skin) significantly at 8 weeks and extremely significantly at 12 weeks, versus the placebo (Figure 7).

The depigmenting efficacy of the verum was stronger on the adjacent zones than on the age spots; the statistically significant performance was found to be approximately 10% stronger on the adjacent zones, after 12 weeks of treatment.



Melanin Index
Age - Spot % Difference versus D0 Value

Figure 6. Melanin Index Age Spots

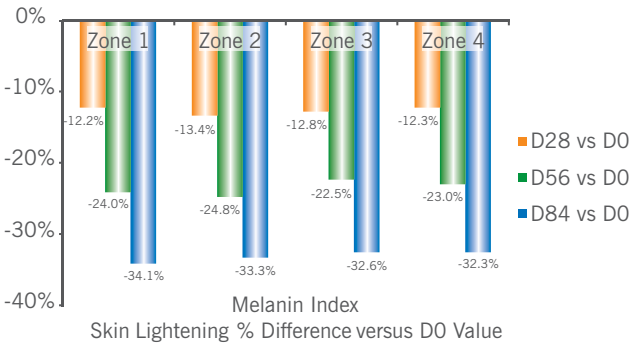


Figure 7. Melanin Index Spot free Zones

The photos (Figure 8) at D0 show irregular pigmented skin on the backs of the hands. The study started at a time (autumn) where a seasonal effect results in less sun exposure which explains the difference between D0 and D84 of the placebo treated hand.

On the treated hand a dark area at D0 (red circle) became significantly lighter at D84. The overall skin colour became much more even and significantly lighter during the treatment with the olive extract.

Conclusion

Skin whitening in Asian countries is an important task of the cosmetic industry. Natural beauty is linked to a pale skin complexion. Skin lightening and age-spot-reduction in Europe and America has become a more and more important subject due to the demographic development of the population. People are becoming older but don't feel old because of an active life, even at a higher age. Looking younger requires fewer wrinkles and an even skin tone. Cosmetic products with active ingredients to fight these signs of ageing are very popular.

In the past, active ingredients like hydroquinone, arbutin and kojic acid dominated the whitening agents' market. Hydroquinone and products containing hydroquinone are still used in cosmetics in certain parts of the world. Current European legislation prohibits their use in cosmetics completely. In the

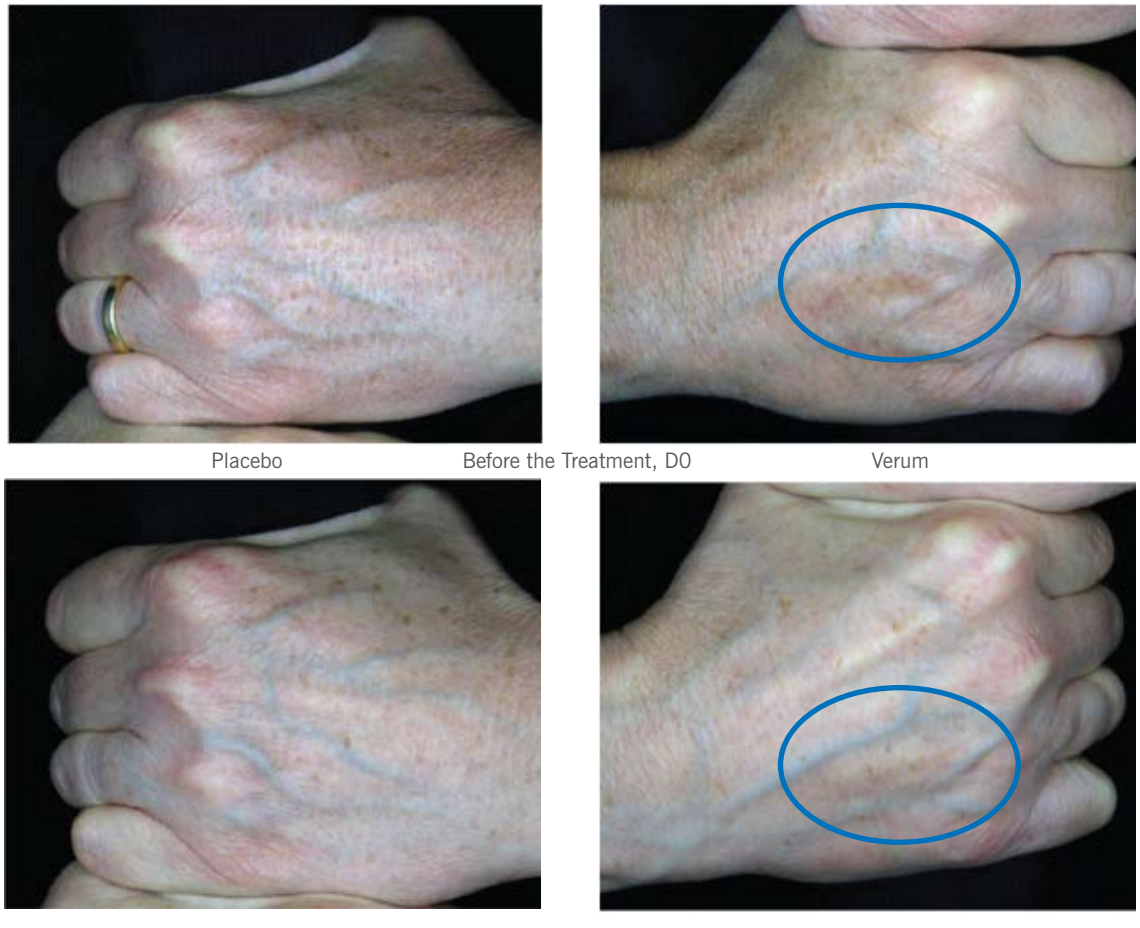


Figure 8. Female Volunteer, Caucasian, 49 years old with very dry skin and Sun Sensitivity (Fitzpatrick) of 2

US hydroquinone is classified as a drug by the FDA. It is no longer approved for use in cosmetics.

Hydroquinone occurs in a variety of forms as a natural product from plants and animals. It has been found in non-volatile extracts of coffee beans and as Arbutin (a glucoside of hydroquinone) in the leaves of blueberry, cranberry, cowberry and bearberry plants[10].

The inherent toxicity of hydroquinone let cosmetic researchers focus on safer natural or nature identical isolates with a similar function, but without the strong side effects. Hydroquinone is known for serious side effects when used over a long period of time[10].

Most skin lighteners currently in use are of botanical or natural origin. Plants with a long history of use are well known to consumers and provide a certain feel of comfort. Olives and olive oil have been known for centuries. Their strong anti-oxidants in the fruits and the leaves have shown interesting anti-ageing effects in skin[2,11]. Hydroxytyrosol as one of the prominent anti-oxidants in olives works in different ways. On the one hand it increases the body's own anti-oxidant power by increasing the amount of glutathione in the cells. An increased amount of glutathione additionally directs the production of melanin towards the more soluble and lighter pheomelanin. On the other hand it lightens skin colour significantly by reducing the overall melanin production[12].

Today effective skin whitening formulations can be developed with safe and natural ingredients. At the same time they protect the skin by activating the body's own defence mechanism.

Note 1: The *in vitro* tests were conducted by CELLnTEC advanced Cell Systems, Bern, Switzerland and the *in vivo* test was conducted by Skin Test Institute, Neuchâtel, Switzerland.

References

1. Informationsgemeinschaft Olivenöl (München): Wissenschaftliche Erkenntnisse über Olivenöl in der Ernährung. Hintergrundinformation Nr. 1 des Instituts für Arteriosklerose Forschung an der Westfälischen-Universität, Münster (2001).
2. D'Angelo S. et al., Hydroxytyrosol, a natural antioxidant from olive oil, prevents protein damage induced by long-wave ultraviolet radiation in melanoma cells, Free Radical Biology and Medicine 1;38(7):908-19, (2005)
3. Maiuri M.C. et al., Hydroxytyrosol, a phenolic compound from virgin olive oil, prevents macrophage activation. Naunyn Schmiedebergs Archives of Pharmacology; 371(6):457-65; (2005)
4. Slominski A., Tobin D.J., Shibahara S., Wortsman J. Melanin Pigmentation in Mammalian Skin and Its Hormonal Regulation, Physiol Rev 84: 1155–1228, (2004)
5. Ebanks J.P., Wickett R.R., Boissy R.E., Mechanisms Regulating Skin Pigmentation: The Rise and Fall of Complexion Coloration, Int. J. Mol. Sci., 10, 4066-4087 (2009)
6. Promega Corporation · 2800 Woods Hollow Road · Madison, WI 53711-5399 USA, GSH-Glo™ Glutathione Assay, Technical Bulletin, (2008)
7. Dawson JB, Barker DJ, Ellis DJ, Grassam E, Cotterill JA, Fisher GW, Feather JW, A theoretical and experimental study of light absorption and scattering by in vivo skin, Phys Med Biol;25(4)695-709, (1980)
8. Verkruysse W., Svaasand L.O., Franco W., Nelson J.S., Remittance at a single wavelength of 390 nm to quantify epidermal melanin concentration, J Biomed Opt. 14(1), (2009)
9. Takiwaki H., Measurement of skin color : practical application and theoretical considerations, The Journal of Medical Investigation Vol.44, (1998)
10. Gillner M., Moore G.S., Cederberg H., Gustafsson K., Hydroquinone, Published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization, (1994)
11. Lueder M., Blank J., Hydroxytyrosol from Olives, an interesting radical scavenger for cosmetic applications, CSC-Conference Amsterdam (2008)
12. Tomohiro C., Whitening effect of hydroxytyrosol containing Oleaceae plants., Fragr. J, Vol.32; (8); 41-48, (2004)

Author's Biography

Maria Lueder holds a degree in chemical engineering specialising in biochemistry, from the University of Applied Sciences in Krefeld, Germany. She began her career as an R&D project manager in Germany and France within the cosmetic industry and then progressed through the area of regional sales responsibility at BFGoodrich, Germany into global application & technical service management at Uniqema, The Netherlands. She moved from a technical position into global marketing responsibilities at Ciba Specialty Chemicals, Switzerland and into a general management position at Med Beauty Trading, Switzerland.

Maria has more than 20 years of experience in the cosmetic industry and has published numerous papers in scientific journals and has made regular presentations at conferences in the area of emulsion technology, polymers and cosmetic actives.

In November 2007 Maria founded her own company Qenax in Zug, Switzerland together with two partners. She is managing the company together with one of the partners, leading R&D and Marketing. Her company is developing and marketing natural cosmetic actives.

In May 2010 Maria Lueder became a board member of the Swiss SCC.