

# NATURAL ANTIOXIDANTS AND THEIR EFFECTS ON THE SKIN

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## 13.1 INTRODUCTION

Antiaging skin care represents a large segment of the cosmetic products market. Such products typically claim to contain the ultimate “miracle in a jar,” that is, a unique active molecule that maintains the youthful aspect of the skin by promoting cellular activity in the epidermis and the dermis. To the best of our knowledge, however, there is no such miracle antiaging compound: the best current approach being to combine antiaging natural antioxidants acting in synergy. “Synergy” means that the global effects of these combined natural antioxidants will be greater than the sum of each one’s specific effects. In this chapter, we will develop the concept how antioxidants play an important role in the prevention of premature aging. We will also address

- why have antioxidants become major ingredients in antiaging cosmetics?
- why are natural antioxidants preferred to synthetic ones by so many brands?
- what are the best approaches to measure antioxidant concentration, efficacy, and long-term effects on the skin?
- what may we learn from the large epidemiological studies on nutrition-based antioxidants that have recently been published?
- what is the relevance of topical application of plant antioxidants (phytoantioxidants), in particular with respect to the outer layer of the skin, the stratum corneum, compared to nutritional antioxidants?

The answers we propose are based on available data and may be subject to change in the future, but they provide a basis for further investigation and pave the way for the optimal use of phytoantioxidants in skin aging prevention.

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## 13.2 OXIDANTS AND ANTIOXIDANTS

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In this section, we will review the basic data on oxidants and their role and function in skin aging.

### 13.2.1 Oxidants

**13.2.1.1 ROS and RNS** Oxygen ( $O_2$ ) is essential to the life of aerobic organisms. However, its metabolites represent a potential threat to all living organisms. Indeed,  $O_2$  is metabolized in animal tissue by successive reductions in superoxide anion ( $O_2^{\bullet-}$ ), hydrogen peroxide ( $H_2O_2$ ), and hydroxyl radical ( $\bullet OH$ ). These different metabolites are called reactive oxygen species (ROS). ROS are either free radicals (with an unpaired electron in their outer orbital sphere) ( $O_2^{\bullet-}$ ,  $\bullet OH$ ) or nonradical ( $H_2O_2$ , singlet oxygen ( $^1O_2$ )) [1]. Nitric oxide ( $NO^{\bullet}$ ), some functions of which overlap with ROS, is synthesized from L-arginine and oxygen by enzymes called NO synthases and is part of the reactive nitrogen species (RNS) [1]. In this chapter, we will essentially focus on ROS.

**13.2.1.2 Physiological Functions** At low concentrations, ROS exert essential intracellular functions, as second messengers, gene regulators, and mediators for cell activation (kinases and transcription factors). They also play a key role in our body's defense against infectious organisms, a role exemplified by the fact that patients with genetic defects of NADPH oxidase, an enzyme involved in ROS production by white blood cells upon infection, are subject to major infections [2]. Furthermore, ROS are modulators of cell death, whether apoptosis or necrosis [1]. In the presence of high concentrations of  $O_2^{\bullet-}$ ,  $NO^{\bullet}$  allows the formation of peroxynitrites that alter mitochondrial membrane potential, which is key to the activation pathways leading to cellular apoptosis [3].

**13.2.1.3 Oxidative Stress** Oxidative stress is defined by an imbalance between ROS and antioxidants, ROS being in excess. Oxidative stress is deleterious to both cells and extracellular matrix, to nuclear and mitochondrial DNA, to membrane lipids, and to proteins. DNA damage (single-strand lesions, deletions of bases, or "cross-links" between DNA and proteins) forms the basis of UV-induced skin carcinogenesis. Lipid peroxidation affects phospholipids both structurally and functionally and results in rigid and permeable membranes. Protein alterations, whether direct or activated by proteases, are reflected in the skin by both reductions in total amount and alterations of collagen and elastin [4]. Moreover, excessive production of ROS or RNS induces mitochondrial damage, leading to a sharp decrease in ATP and cell death by necrosis [5].

**13.2.1.4 UV Exposure as the Major Source of Oxidative Stress in the Skin** UV exposure (180–400 nm) leads to multiple cellular damages, by generating  $^1O_2$ ,  $\bullet OH$ ,  $H_2O_2$ , and other ROS. UVB rays (290–320 nm) are absorbed by epidermal chromophores such as melanin and urocanic acid and lead to direct molecular damages while also generating ROS. In the presence of  $H_2O_2$ ,

UVB radiation leads to the formation of  $\cdot\text{OH}$  [6], which causes DNA damages. UVA rays (320–400 nm) penetrate more deeply in the dermis, increase the production of ROS, and contribute to long-term actinic damage. Both UVA and UVB induce the activation of a wide range of transcription factors in skin cells, including NF- $\kappa$ B (transcription factor involved in inflammation and cellular stress responses) [7], which in turn may increase the production of matrix metalloproteinases (MMPs), a family of enzymes that degrade collagen and elastin.

The skin is continuously affected by environmental factors and notably UV radiation [8]. In the skin, free radicals induced by UV radiation cause damage to DNA, to proteins, and destabilize the membranes of keratinocytes, leading to premature aging of the skin cells: “oxidation = aging.” When exposed to UV radiation, the skin undergoes alterations resulting in inflammation, photoaging, and various skin disorders [9]. Skin photoaging is accompanied by wrinkling, loss of elasticity, increased skin fragility, and slower wound healing.

### 13.2.2 Antioxidants

Living tissues have a control mechanism to keep ROS in balance. When ROS are generated *in vivo*, many antioxidants come into play. Their relative importance depends upon which ROS are generated, how and where they are generated, and which target of damage is considered [1]. Our body defends itself from these phenomena via endogenous antioxidants [1, 10, 11]. However, when endogenous antioxidants become insufficient or imbalanced in defense against oxidants, exogenous antioxidants may help restore the balance.

Antioxidants inhibit the production of ROS by direct scavenging, decrease the amount of oxidants in and around our cells, prevent ROS from reaching their biological targets, limit the propagation of oxidants such as the one that occurs during lipid peroxidation, and thwart oxidative stress thereby preventing the aging phenomenon.

**13.2.2.1 Endogenous Antioxidants** Endogenous antioxidants are essentially enzymes that catalytically remove oxidants. Major endogenous antioxidants are superoxide dismutase, superoxide reductase, catalase, and glutathione peroxidase. These enzymes play a key role in decreasing the content of oxidants and preventing oxidative damage. Other endogenous antioxidant molecules, such as heme oxygenase, minimize the availability of oxidants. This enzyme is strongly induced by oxidative stress and removes an oxidant (heme) while generating a putative antioxidant (bilirubin that is sensible to  $^1\text{O}_2$ ) and a prooxidant (iron). In addition, high ferritin levels result in an increased iron scavenging capacity that may confer increased resistance to oxidative stress [12].

The levels and composition of endogenous antioxidant molecules differ from tissue to tissue and by cell type. Both embryonic and adult stem cells express high levels of antioxidant enzymes, which decrease as the cells differentiate [13]. The endogenous antioxidant molecules are often increased after exposure to oxidants [1]. However, this “antioxidant pool” is gradually consumed, as oxidant exposure increases over time.

**13.2.2.2 Exogenous Antioxidants** Exogenous antioxidants include antioxidants that cannot be synthesized by our body such as vitamins, trace elements, and phytoantioxidants. Vitamin E (tocopherol) is the most powerful liposoluble antioxidant. It inhibits the peroxidation of membrane lipids. It reacts with free radicals to form the radical tocopheryl, a stable substance that stops the chain reaction of the membrane lipids. The chain reaction is propagation of free radicals: the molecules destabilized by a single electron in turn become free radicals that remove an electron from another molecule that thus becomes a radical, and so on. Tocopheryl has the ability to stop this type of chain reaction by membrane lipids. It works in conjunction with other antioxidants such as vitamin C and selenium. Vitamin C is a water-soluble vitamin and has a strong antioxidant activity that protects cells against damage by free radicals. This vitamin reacts with the tocopheryl radical to regenerate and restore vitamin E. It then becomes the ascorbyl radical, which is also relatively stable. Trace elements such as selenium are important cofactors of the activity of antioxidant enzymes.

**13.2.2.3 Food-Derived Antioxidants** In 1992, Serge Renaud, professor at the University of Bordeaux, proposed as “the French paradox” the apparent contradiction between the nutrition of French men and their health [14]. In the Southwest of France, food is fairly rich in fat and wine is readily consumed, yet the overall health of the population is quite good, the prevalence of cardiac infarction lower than in the United States, and life expectancy is higher than in the Northeast of France. The proposed explanation for this phenomenon is the high consumption of polyphenols in red wine consumed by the inhabitants of Southwestern France [15]. Polyphenols in red wine were found to inhibit low-density lipoprotein (LDL) oxidation *in vitro*, and it was suggested that they could exert cardioprotective effects by limiting LDL oxidation *in vivo*.

The SUVIMAX (SUPplementation en Vitamines et Mineraux AntioXidants) study examined 7876 French women aged 35–60 years and 5141 men aged 45–60 years (all apparently healthy) for more than 7 years (1994–2002). Daily, they were given either a placebo or a pill containing 120 mg vitamin C, 30 mg  $\alpha$ -tocopherol, 6 mg  $\beta$ -carotene, 100  $\mu$ g selenium, and 20 mg zinc. This low-dose supplementation had no significant effect on incidence of cancer or cardiovascular disease for the group as a whole. However, for men only, there was a preventive effect for skin and lung cancer. It also appears that diets rich in a variety of phytoantioxidants (fruits, grains, and vegetables) are protective against several human diseases; hence the nutritional worldwide program: eat five servings of fruits and vegetables everyday!

Because the risk of oxidative stress increases with age, and because endogenous antioxidants are gradually consumed over time, prevention strategies are essential. Natural antioxidants play key roles in these strategies (Section 13.3). The skin, as a cutaneous barrier, is constantly subjected to damage from the environment. As such, it consumes its endogenous antioxidants that may be replenished by exogenous antioxidants provided topically (Section 13.4). Also, specific nutritional factors were found to upregulate endogenous antioxidants.

### 13.3 NATURAL ANTIOXIDANTS (PHYTOANTIOXIDANTS)

Plants suffer from oxidative stress induced by UV radiation as much as animals and humans do, but cannot protect themselves as humans do by exogenous means and have therefore developed multiple strategies and highly effective molecules to defend themselves against environmental stress. For example, edelweiss or lichens contain natural substances that absorb UVB and act as a “screen” [16, 17]. Plants contain multiple antioxidants effective in ideal combinations, the so-called phytoantioxidants, capable of both protecting their own cells and extracellular matrix against oxidative stress induced by UV radiation and of conferring protection to other organisms upon ingestion or topical application.

Most phytoantioxidants belong either to polyphenols or terpenes and form a family of multiple factors from multiple plants (Figure 13.1). Polyphenols are synthesized by plants, participate in their metabolism, and contribute to their defense against environmental stresses. Polyphenols are found in roots, stems, flowers, and leaves of all plants. They differ among themselves by molecular weight, polarity, and solubility. Polyphenols contain an —OH group attached to a benzene ring. The number of phenolic —OH groups and their relative positions are key determinants of polyphenols’ antioxidant activity: these phenolic groups exert direct antioxidant effects, modulate protein phosphorylation, and inhibit lipid peroxidation by acting as chain-breaking peroxy radical scavengers. The large family of flavonoids, stilbens, and terpenes (Table 13.1) help to prevent cellular and extracellular oxidative stress and slow the aging of the skin; the carotenoids do so more specifically by quenching singlet oxygen ( $^1\text{O}_2$ ). More than 4000 distinct flavonoids have been identified, the most important being anthocyanidins, flavanols, isoflavones, and flavanones (Table 13.1).

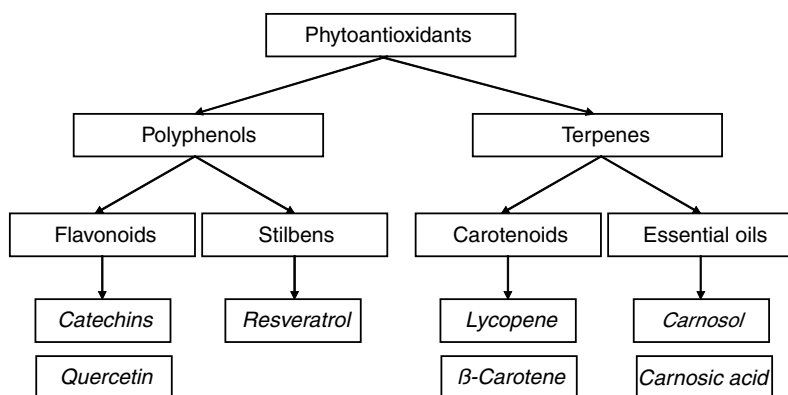


Figure 13.1 Phytoantioxidants: a family. Phytoantioxidants represent large family of molecules: polyphenols and terpenes with subcategories including flavonoids, stilbens, carotenoids, and essential oils. Some examples of phytoantioxidants described in this chapter are shown in italics (adapted from Ref. [50]).

**TABLE 13.1 Three Families of Phytoantioxidants with Respective Sources and Effects**

Flavonoids	Anthocyanidins	Anthocyanins	Present in blueberries, basil grapes; powerful scavengers of free radicals; inhibit lipid peroxidation	Ref. [51]
	Flavanols	Catechins	Present in green tea, grape seeds, litchi; inhibit the production of H <sub>2</sub> O <sub>2</sub> induced by UV; protect the endogenous antioxidant systems	Ref. [52]
	Flavonols	Quercetin	Present in green tea; blueberries, grape seeds, apple; protects the antioxidant systems in the skin	Ref. [44]
	Isoflavones	Genistein	Present in ginkgo biloba, soja; increases the activities of endogenous antioxidants	Refs [52, 53]
	Flavanones	Silymarin	Present in milk thistle; inhibits lipid peroxidation; promotes cell regeneration	Ref. [54]
Stilbens	Resveratrol		Present in grapes, berries; inhibits the production of H <sub>2</sub> O <sub>2</sub> and lipid peroxidation	Ref. [54]
Terpenes	Carotenoids	Lycopene	Neutralizes <sup>1</sup> O <sub>2</sub> ; scavenges the lipid free radicals; reduces lipid peroxidation	Ref. [55]

## 13.4 MEASURING ANTIOXIDANT CAPACITY

Phytoantioxidants are comprised of thousands of molecules with different and complementary antioxidant properties, including free radical scavengers, antioxidant defense mechanism elicitors, iron chelators, light and UV physical screens, direct or indirect repair systems. One of their advantages lies in their combined antioxidant properties. Here, we will focus on the methods available for determining their antioxidant capacity. This can be done by analytical methods or by measuring the effects of antioxidants, either directly or indirectly, for example, by analyzing the degree of protection from UV radiation they induce.

### 13.4.1 Quantification of Free Radicals

As phytoantioxidants scavenge free radicals, the quantification of free radicals can be used for monitoring their protective properties. Such techniques are based on detection of unpaired electrons by spectroscopy. The direct measurement of free radicals is difficult because their half-life is extremely short. •OH, for example, has a half-life of 10<sup>-6</sup> s and a diffusion distance of 10 nm. Electron spin resonance

(ESR), however, enables the direct measurement of radical generation; the use of cold temperatures may contribute to more effective measurements [18]. The direct detection of free radical formation in human and animal skin following exposure to UV radiation has been obtained by low-temperature ( $-196^{\circ}\text{C}$ ) ESR spectroscopy *in vitro*. These approaches are not used extensively in routine measurements because of their complexity and cost.

### 13.4.2 Quantification of Damage Markers

The protective role of phytoantioxidants can also be measured by using different techniques of quantification of specific end products resulting from the interactions of free radicals with target molecules such as DNA, lipids, metabolite intermediates, or reporter molecules. Once the quantification of the damage markers is established, the extent of protection provided by antioxidants can be assessed. However, such assays require the use of heavy equipment in a laboratory, as well as expert technicians, are essentially used for indirect measurement of oral uptake of antioxidant-rich food, antioxidant-rich supplements, and/or exposure to hazardous chemicals, and include the following:

- **The 8-hydroxy-2'-deoxyguanosine (8OHdG) assay.** In the case of DNA oxidative stress, guanosine is preferentially oxidized and is used as a biomarker. 8OHdG is detected by standard methods such as high-performance chromatography (HPLC). For example, dermal uptake of polycyclic aromatic hydrocarbons containing oil was shown to increase the urinary production of 8OHdG [19].
- **The Comet assay.** This assay is based on gel electrophoresis of DNA molecules. Intact and damaged DNA display different migrating properties. Damaged DNA displays a “comet”-like trail and the higher the damage the longer the trail. Modifications of the Comet assay have been developed so that the DNA repair capacity can also be monitored. This assay has been used to monitor a variety of conditions that modify DNA, including dietary protective factors [20, 21]. Morley et al. used the Comet assay to investigate green tea’s photoprotective effects by comparing DNA damage induced by UV exposure in cultured human cells in the presence or absence of green tea [22].
- Thiobarbituric acid reactive substances (TBARS) and malonaldehyde (MDA) are used as biomarkers of lipid oxidation and were used for measuring the photoprotective role of a rosmarinic acid extract both *in vitro* and *in vivo*, after oral intake [23].
- The isoprostanes assay is considered as one of the most reliable methods for the evaluation of oxidative stress *in vivo* [24]. Isoprostanes are prostaglandin-like products formed *in vivo* from the peroxidation of arachidonic acid [25]. Their dosage has been successfully used to analyze photooxidative UVB-induced skin damage *in vivo*, and a linear correlation between the UVB exposure and the generation of 8-isoprostanes was found [26].

### 13.4.3 Quantification of Antioxidant Capacity

Phytoantioxidants include several different active principles. Some of these active principles can be purified and measured separately by standard analytical methods. When measuring the effects of an isolated compound, however, the positive or negative modulation of its activity by the biochemical environment is dismissed. Therefore, various assays have been developed to measure the global antioxidant activity, so that these interactions can be taken into account. They can be classified according to the chemical reactions involved, whether electron transfer (ET-based assays) or the hydrogen atom transfer (HAT). These assays, as discussed below, are used for the quantification of the antioxidant capacity of antioxidant-rich food, plant extracts, and biological fluids. Electrochemical-based assays appear promising in the evaluation of complex phytoantioxidants and the measurement of their skin-protective properties.

**13.4.3.1 ET-Based Assays** ET assays are based on antioxidants' reducing capacity by involving one redox reaction. Such methods include the following:

- The 2,2'-diphenyl-1,1'-picrylhydrazyl (DPPH) assay, where the decrease in absorbance of DPPH is proportional to the concentration of free radical scavengers.
- The trolox equivalent antioxidant capacity (TEAC) assay, where the interactions between antioxidants and 2,2' azinobis(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) result in the ABTS change of color (from blue green to colorless).

**13.4.3.2 HAT-Based Assays** The HAT-based assays involve a synthetic free radical generator, an oxidizable molecular probe, and the antioxidant to be tested. The antioxidant capacity depends on the capacity of donating hydrogen atoms. Such methods include the oxygen radical absorbance capacity (ORAC) and the total radical trapping antioxidant parameter (TRAP) assays. The ORAC assay measures the antioxidant inhibition of free radical damage to a fluorescent probe over time, as compared to Trolox, a water-soluble analogue of  $\alpha$ -tocopherol [27]. This is particularly suited to measuring slow and fast acting antioxidants in foods and plant extracts (Figure 13.2) [28]. The TRAP assay is based on the properties of "azo-initiators" resulting in the production of a peroxy radical that can initiate a peroxidation chain reaction [29, 30].

**13.4.3.3 Electrochemical-Based Assays** Electrochemical-based assays are used to determine the redox capacity of single or complex compounds. Cyclic voltametry techniques have been adapted to the quantification of the overall reducing capacity of low molecular weight antioxidants in different biological fluids [31]. Such assays do not require the use of reagents and do not depend on absorbance. They do, however, depend on interactions taking place between the tested samples and the electrode surface. The recently developed EDEL assay (EDEL Therapeutics, Switzerland) is based on the titration of an ideal and virtual antioxidant

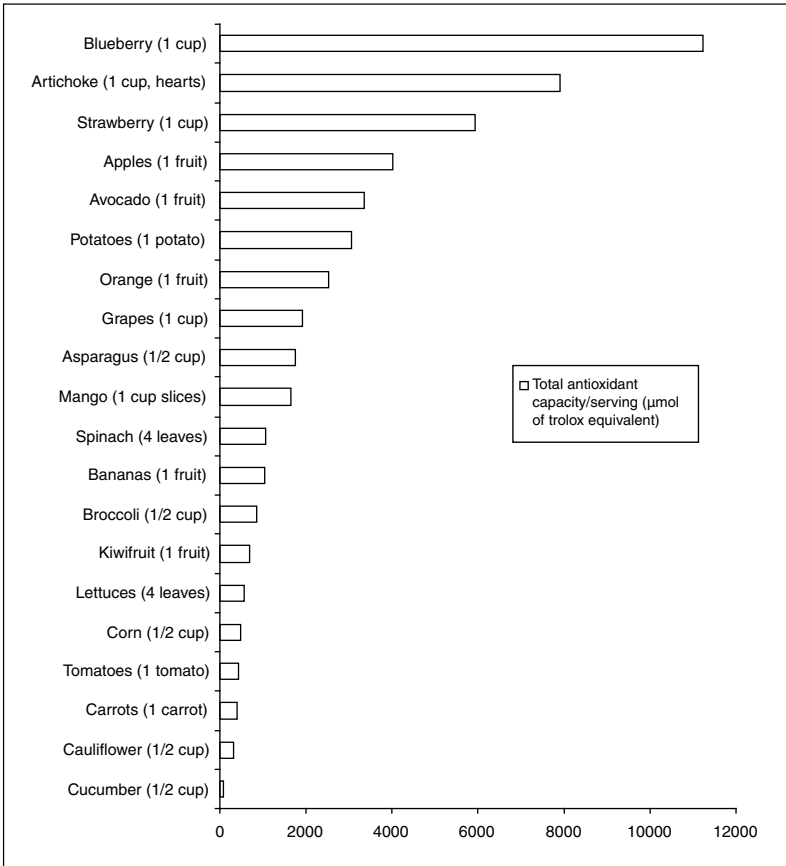


Figure 13.2 ORAC results of different fruits and vegetables. A higher ORAC value represents a more powerful antioxidant activity. Of those tested here, blueberry is the most powerful antioxidant with an ORAC value of 11,400 (adapted from Ref. [28]).

(pending patent number WO/2009/039945), which can be used for measuring a variety of samples (products, cosmetics, biological fluids, and skin) in less than 20 s. This procedure does not require costly or heavy equipment (the electrochemical unit, used in combination with a computer, is portable) and may be applied repeatedly to various biological samples, allowing for direct comparisons. The samples can be analyzed on site upon collection, there is no need for reagents, and single-use strips prevent the risk of cross-contamination. This method allows for *in vitro* antioxidant activity measurements of different cosmetic formulations and their *in vivo* effects on the skin. For *in vivo* measurements, the specially designed well containing PBS is applied to the forehead for 5 min, the required time to extract antioxidants of the skin in the PBS. The antioxidants released in the PBS may then be measured by the electrode in the supernatant. This procedure can be used before and after the application of a protective formulation on the skin (Figure 13.3a and b). The impact of different treatments can also be measured, that is, UV and/or environmental

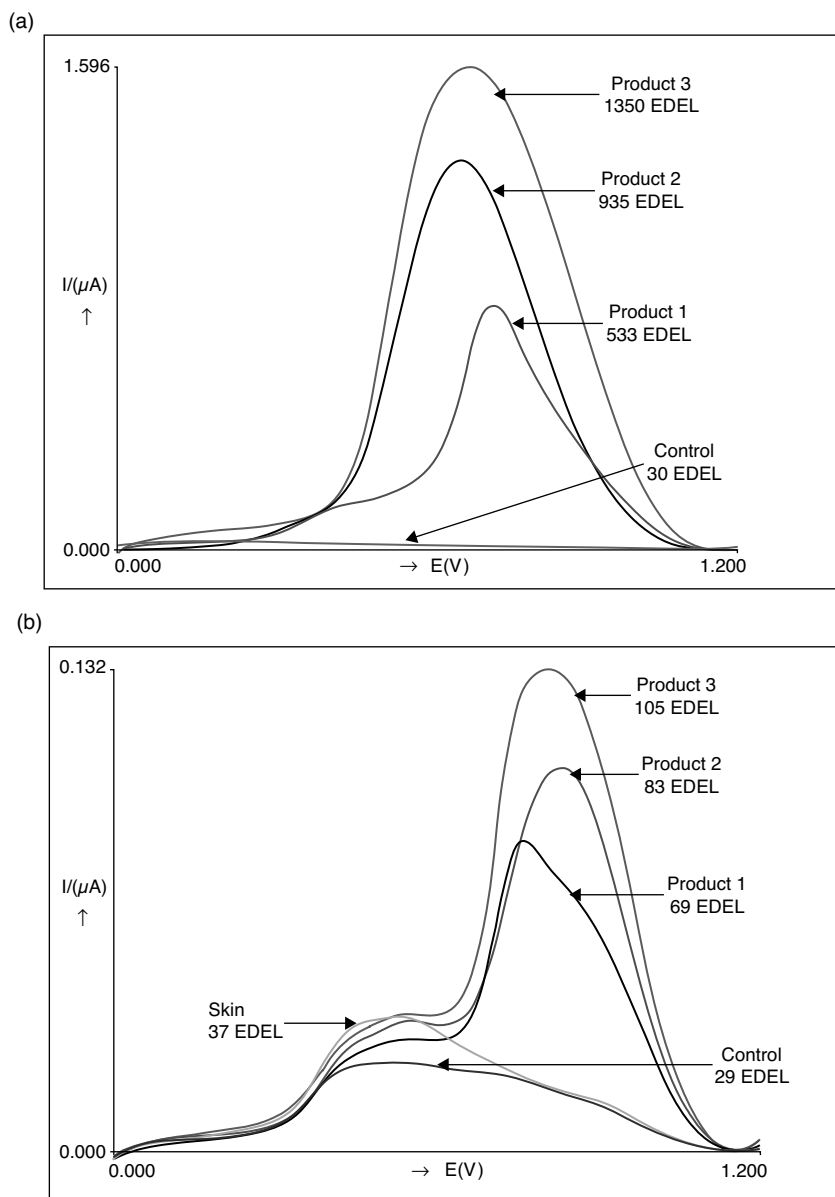


Figure 13.3 Antioxidant activity of three cosmetic products *in vitro* and *in vivo*. (a) Antioxidant results *in vitro* of three cosmetic products in their jars compared to a control solution. Three different cosmetic formulas claiming antioxidant effects were analysed by electrochemistry with Edelscan (Edeltherapeutics, Switzerland). Results are the mean of triplicate measurements. The control is phosphate buffer solution (PBS). (b) Antioxidant results *in vivo* of the same cosmetic products on the skin. 100  $\mu\text{L}$  of PBS was used to extract the skin's antioxidants in 5 min, using an especially made well, on a 314  $\text{mm}^2$  area. Two milligrams of product were applied to the skin for 10 min. The skin's antioxidants were measured by electrochemistry with Edelscan before and after the application of three similar cosmetic formulas. Results are the mean of triplicate measurements. Before product application, the skin contains endogenous antioxidants, and after product application, the level of skin's antioxidants increased.

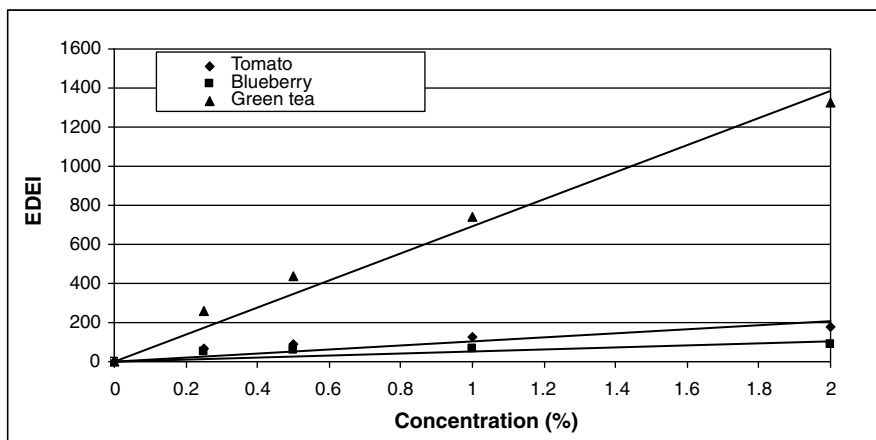


Figure 13.4 Antioxidant activity of three natural extracts *in vitro* as a function of their concentration. A stock solution of 10% w/w of green tea extract, 5% w/w of blueberry extract, and 0.5% w/w of tomato extract each was prepared in phosphate buffer, pH 7.4 (PBS). Each solution was further diluted 50%, 25%, and 12.5%, respectively, into PBS and the antioxidant capacity measured immediately by electrochemistry with Edelscan (Edeltherapeutics, Switzerland). Results are the mean of triplicate measurements. Green tea presents a higher antioxidant activity than tomato or blueberry.

pollution exposure. Figure 13.4 shows the results obtained with three different natural ingredients: green tea, tomato, and blueberry extracts, all contain powerful phytoantioxidants and serve as references. The ability to measure the antioxidant properties of phytoantioxidants and their biological effects will improve the selection of active ingredients, as well as the manufacturing processes, quality control, and packaging of cosmetic natural formulations, and will complement the clinical studies on the effects of antioxidants to determine the most effective antioxidant strategies.

## 13.5 CLINICAL STUDIES OF NUTRITIONAL AND TOPICAL ANTIOXIDANTS

### 13.5.1 Effects of Nutritional Supplements on Skin

The effects of antioxidants on cancer and cardiovascular diseases are well described. But what about their effects on the skin? Brosche and Platt showed that consumption of borage oil improves the cutaneous barrier function in elderly individuals, illustrated by a decrease in transepidermal water loss (TEWL) [32]. Specific nutritional factors favor skin hydration, elasticity, and sebum production, and stimulate the physiological properties of the skin [33–35]. Supplementation with vitamin E, vitamin C, and/or carotenoids was shown to provide protection against UV radiation, although the sun protection factor was relatively small compared to topical sunscreens [34].

### 13.5.2 Effects of Dietary Antioxidant Supplementation on the Skin

Several clinical and laboratory studies have recently investigated the effects on the skin of dietary antioxidant supplementation. Here, we report the results of three of these studies, highlighting their particularly interesting conclusions.

**13.5.2.1 Study on Epigallocatechin Gallate Supplementation [36]** This study investigated the effects of oral epigallocatechin gallate, a powerful antioxidant in green tea, on the minimal erythema dose and UV-induced skin damage. Female hairless rats were fed a normal diet supplemented with 1.5 ppm epigallocatechin gallate for 8 weeks. The minimal erythema dose was determined and visual scores and transepidermal water loss were assessed to evaluate the severity of UV-induced skin damage. At week 8 of the study, the consumption of epigallocatechin gallate increased the minimal erythema dose significantly. UV radiation-induced sunburn severity alterations in epidermal barrier function were also attenuated by the supplementation of epigallocatechin gallate. The regular intake of epigallocatechin gallate strengthens the skin's tolerance by increasing the minimal erythema dose and thus prevents UV-induced perturbation of epidermal barrier function and skin damage.

**13.5.2.2 Study on Supplementation with a Mixture of Various Antioxidants [37]** Thirty-nine volunteers with healthy type II skin were divided into three groups ( $n = 13$ ) and supplemented for a period of 12 weeks. Group 1 received a mixture of lycopene (3 mg/day), lutein (3 mg/day),  $\beta$ -carotene (4.8 mg/day),  $\alpha$ -tocopherol (10 mg/day), and selenium (75 mg/day). Group 2 was supplemented with a mixture of lycopene (6 mg/day),  $\beta$ -carotene (4.8 mg/day),  $\alpha$ -tocopherol (10 mg/day), and selenium (75 mg/day). Group 3 was the placebo group. Skin density and thickness were determined by ultrasound measurements. Skin roughness and scaling were decreased by supplementation with antioxidant micronutrients. In the placebo group, no change was found in any of the parameters.

**13.5.2.3 Study on  $\beta$ -Carotene Supplementation [38]** Sixteen healthy women were given a dosage of  $\beta$ -carotene (30 mg/day) for 10 weeks. After the 10-week supplementation period, supplementation continued with exposure to natural sunlight for 13 days. During this period, the development of erythema in subjects who had taken  $\beta$ -carotene was much less pronounced than in the placebo group. Supplementation with  $\beta$ -carotene (30 mg/day) before and during sunlight exposure provided protection against sunburn.

These studies indicate that oral antioxidant supplementation may protect against UV-induced skin damage and improves the quality of the skin. How does topical application of antioxidant-rich cosmetics compare to nutritional supplementation?

### 13.5.3 Effects of Topical Antioxidants on the Skin

Green tea, rosemary, grapes, and tomato are four classical examples of plants that are most studied for their direct antioxidant activity on the skin and skin cells *in vivo* and *in vitro* and serve as a reference for other fruits and vegetables.

**13.5.3.1 Green Tea** Green tea contains four major flavonoids: epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin-3-gallate. These molecules have the ability to scavenge ROS:  $O_2^{\bullet-}$ ,  $\bullet OH$ ,  $H_2O_2$ , and  $^1O_2$ . Katiyar et al. have shown that epigallocatechin applied topically on mice exposed to UVB inhibits the production of  $H_2O_2$  both in the dermis and in the epidermis [39].

**13.5.3.2 Rosemary** Rosemary contains various antioxidants, in particular phenolic diterpenes: carnosol and carnosic acid represent over 90% of the antioxidant properties of rosemary extract [40]. These lipophilic molecules scavenge lipid free radicals, thereby enabling the reduction of lipid peroxidation and inhibiting oxidative damages to skin surface lipids [41]. Pretreatment of human fibroblasts with carnosic acid resulted in the suppression of metalloproteinase-1 messenger RNA elevation caused by UVA irradiation. Carnosic acid also has photoprotective potential [42].

**13.5.3.3 Grape Seeds** Grape seeds are major sources of resveratrol and quercetin. The stilben resveratrol inhibits lipid peroxidation induced by UVB and significantly decreases UVB-induced skin thickness and edema in hairless mice [43]. The iron chelator flavonoid quercetin maintains and protects the activities of glutathione peroxidase, catalase, and superoxide dismutase after exposure to UV radiation [44].

**13.5.3.4 Tomato** Tomato is rich in lycopene, a widely studied powerful antioxidant and anticarcinogenic carotenoid with strong reducing ability and the most effective carotenoid in the capture of  $^1O_2$ . Lycopene scavenges lipid radicals, reduces lipid peroxidation, and prevents erythema caused by UV radiation on the skin [45].

## 13.6 FROM THE PLATE TO THE JAR TO THE STRATUM CORNEUM

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As dietary antioxidant intakes prevent aging effects on various organs including the skin, one could question why it is so important to apply phytoantioxidants to the upper layer of the skin, the stratum corneum. The fact is that the local production of ROS upon exposure to UV radiation and/or other environmental hazards requires topical application of antioxidants in order to optimally prevent ROS-induced local skin damage.

### 13.6.1 The Stratum Corneum: Structure and Function

The stratum corneum is the outermost layer of the skin. It is 10–20  $\mu m$  thick, although this thickness varies from a few micrometers to millimeters depending on body parts. It contains only 15% water (whereas the whole body contains 65% and the heart 76% water) and consists of two distinct structural components: the corneocytes and the intercellular lipids. Elias proposed the “brick and mortar model,” according to which

the stratum corneum is composed of flat cells (bricks) surrounded by a lipid matrix (mortar) [46].

The stratum corneum represents only 10% of the entire skin but contributes to over 80% of the cutaneous barrier function; it prevents the loss of water from the epidermis and provides protection from the outside environment through its antioxidants.

The stratum corneum, due to its critical location at the interface between the body and the environment, is continuously exposed to oxidants, including UV radiation, chemical oxidants, air pollutants, and microorganisms. The stratum corneum is a paradoxical tissue composed of nondividing cells, remaining a metabolically active tissue, able to protect itself, to communicate, and to exchange [47]. The stratum corneum, similar to other human living tissue, contains a number of endogenous antioxidant enzymes, such as catalase, superoxide dismutase, and glutathione peroxidase [48].

### 13.6.2 The Stratum Corneum as Target for Topical Phytoantioxidants

The stratum corneum is a prime target for cosmetologists for investigating the penetration and efficacy of cosmetic products. Numerous *in vitro* studies have been conducted to demonstrate the penetration of cosmetic ingredients and their effects on the structure and metabolism of skin cells. It is, however, not yet clear as to whether there is and if so to which extent *in vivo* penetration of compounds from cosmetic formulations. Furthermore, the lack of penetration of the products' active ingredients could very well not be a negative characteristic. Indeed, these ingredients are still able to protect the stratum corneum against oxidative and other environmental damages.

The stratum corneum requires antioxidants to protect itself from the environment.  $\alpha$ -Tocopherol on the one hand accumulates in the lower part of the stratum corneum, providing protection against lipid peroxidation and allows stabilization of lipid bilayers. Phytoantioxidants on the other hand neutralize UV-induced oxidation of the stratum corneum and provide protection from the environment. The use of phytoantioxidants in cosmetic products may stimulate the stratum corneum to regenerate, to protect itself—and thus the underlying epidermis and dermis—from the harmful effects of UV and other environmental toxins, and nourish the skin in the same way as eating fruits and vegetables nourishes the whole body: “from the plate to the jar.”

## 13.7 MULTIPLE PHYTOANTIOXIDANTS IN COSMETICS: A CASE STUDY

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What are the links between all these data and the current world of cosmetics and how does science influence formulation? In order to address this issue, an example of the use of cosmetic formulation and the effects of phytoantioxidants will be discussed.

In 1999, we formulated, according to Swiss quality standards (SwissCos [49]), the serums Diode1<sup>®</sup> and Diode2<sup>®</sup> for women (Alchimie Forever, Geneva, Switzerland). These serums contain high concentrations of antioxidant molecules, have antiaging properties, and contribute to the fading and prevention of brown spots. These serums are presented in two bottles: the first bottle, Diode1<sup>®</sup>, contains water, cellulose gum, chlorphenesin and methylparaben, and 5% of rosemary extract from Morocco (provided by the Swiss supplier Cosmetochem), containing many antioxidant molecules such as terpene phenolics, carnosic acid, and carnosol that have the ability to scavenge lipid free radicals, reduce lipid peroxidation, and have anti-inflammatory properties (Section 13.5.3.2) [41]; the second bottle, called Diode2<sup>®</sup>, contains water, cellulose gum, chlorphenesin, and methylparaben, and 10% of green tea extract from China (provided by the Swiss supplier Botanica). The main antioxidant polyphenols in green tea are catechins, epicatechins, and their derivatives (Section 13.5.3.1). The antioxidant and healing properties of green tea have been studied in detail in the skin [39]. Measurements performed by EDEL Therapeutics were used to document *in vitro* and *in vivo* antioxidant properties of the serums Diode1<sup>®</sup> and Diode2<sup>®</sup>. The results are shown in Figure 13.5a and b. This figure shows that the two serums exert an antioxidant activity that is measurable both in the bottle and after application on the skin, indicating persistent antioxidant activity *in vivo*. These types of measurements should be generalized to confirm the claimed antioxidant effects of skin care products and allow comparisons among them.

## 13.8 CONCLUSIONS AND PERSPECTIVES

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In this chapter, we discussed a number of topics including why antioxidants have become major ingredients in antiaging cosmetics and why natural antioxidants are preferred to synthetic ones by a number of brands. We defined oxidative stress as an imbalance between ROS and antioxidants. In the skin, excess free radicals induced by UV cause damage to cellular macromolecules and destabilize the membranes of keratinocytes, leading to premature aging. Our body defends itself from these phenomena via endogenous and exogenous antioxidants. Natural antioxidants play key roles in these strategies as plants have developed multiple strategies to cope with environmental insults. They generate highly effective molecules to defend themselves against environmental stress. From the large epidemiological studies that have been published recently on nutrition-based antioxidants, we learned that specific nutritional factors favor skin hydration, elasticity, and sebum production, and stimulate the physiological properties of the skin and that oral antioxidant supplementation may protect against UV-induced skin damage.

Current thinking is that a combination of different phytoantioxidants would be the best defense strategy against ROS. We also detailed the best approaches to measure antioxidants and their effects, which will lead to improved cosmetic formulation for the prevention of premature skin aging.

With respect to topical application of phytoantioxidants, we conclude that the stratum corneum is a prime target for cosmetic formulators, as it requires the contribution of antioxidants to protect itself from the environment. Phytoantioxidants

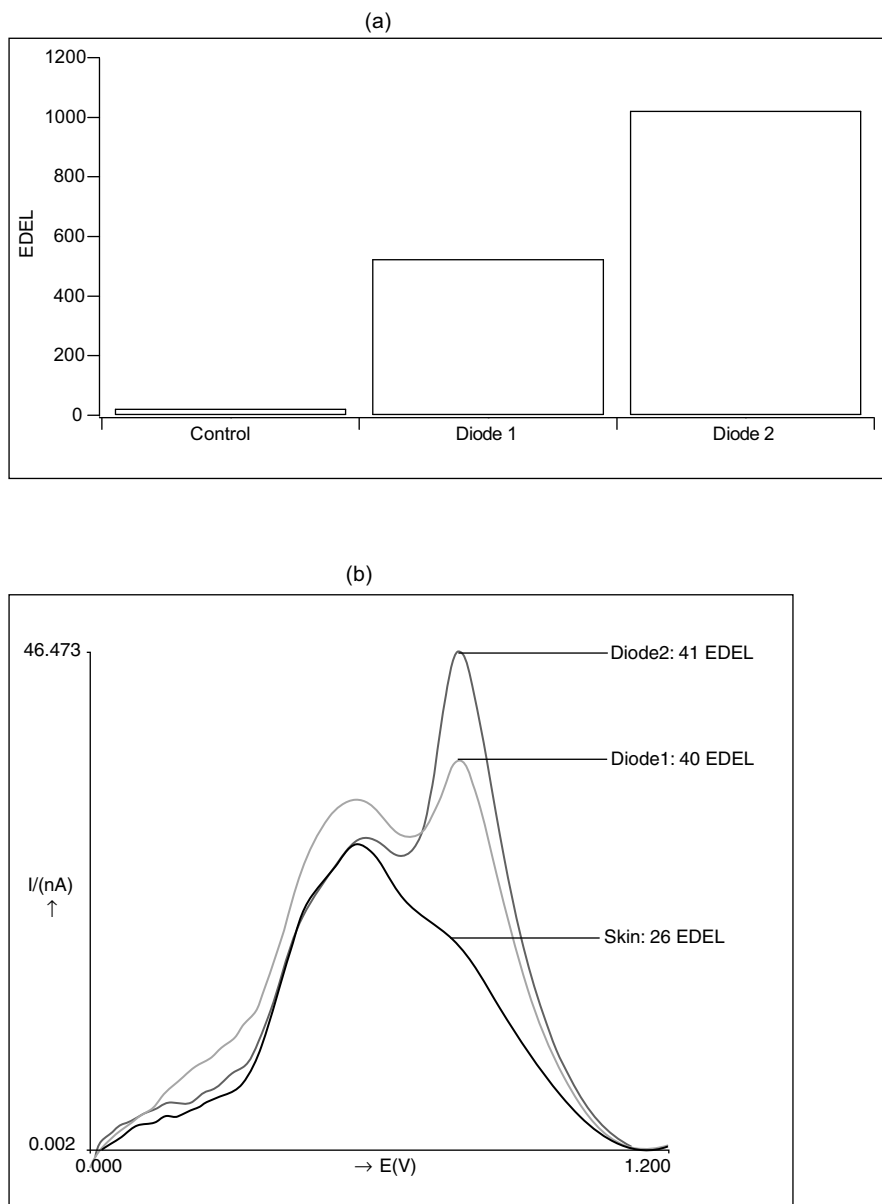


Figure 13.5 Antioxidant activity of Diode1<sup>®</sup> and Diode2<sup>®</sup> *in vitro* and *in vivo*. (a) Antioxidant results *in vitro* of Diode1<sup>®</sup> rosemary and Diode2<sup>®</sup> green tea serums. The green tea serum displays a higher antioxidant activity than the rosemary serum. (b) Antioxidant results *in vivo* of Diode1<sup>®</sup> rosemary and Diode2<sup>®</sup> green tea serums measured directly on the forehead. The antioxidant activity of rosemary and green tea persists once applied on the skin with again a higher activity of the green tea.

neutralize UV-induced oxidation of the stratum corneum, provide protection from the environment, and in cosmetic products may stimulate the stratum corneum to regenerate. Phytoantioxidants are therefore to be used both topically and orally and should be integrated into any antiaging strategy. The use of phytoantioxidants in cosmetic products enables one to nourish the skin and replenish it in antioxidants in the same way as we nourish our body by eating fruits and vegetables.

Further studies along these lines, in particular on the antioxidant capacity of cosmetic formulas *in vivo*, will pave the way for optimal science-based natural health, well-being, and youthful skin.

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