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How Active are Biocosmetic Ingredients ?

Keywords: antioxidants, free radicals, anti-aging

Abstract

The reaction between an antioxidant and free radicals is characterized mainly by the reaction capacity, thus the amount of free radicals neutralized, and the reactivity, thus the velocity of the reaction process. Both parameters can be evaluated simultaneously by the Antioxidative Power (AP) method, able to analyse all the different classes of antioxidants. Herein we present the AP values and the reaction times of several antioxidants used in modern cosmetic skin and hair care products. The knowledge about the reactivity and stability of antioxidants can help to create effective anti-aging and skin/hair care products.

Introduction

Anti-aging products are the most requested class of cosmetics at this time. This constant growing market requests new and active ingredients and customers require an efficacy proved product. A nationwide market survey conducted in the USA among 1008 men and women has shown that 63% of the respondents view the presence of the antioxidant vitamins E and C, in skin care and sun care products as important factor in their purchasing decision (1). Also bioingredients as herb extracts and coenzymes have a growing importance in modern skin and hair care. Most of the modern skin care products contain α-tocopherol or its esters (acetate, palmitate), flavonoids in the form of rosemary, ginkgo, sunflower, grape seed oil, or green tea extracts, and ascorbyl palmitate.

The mentioned active ingredients are expected to fight against a wide-range spectrum of aging signs, as wrinkling, skin drying, photoaging, and pigmentation. These biological effects are caused by very different biochemical processes inside the skin and the beneficial effect of a topically applied substance is often difficult to measure and to quantify. But one common effect all antioxidants should have is the reduction of free radical reactions. Free radicals are the first and common cause of skin aging, in particular photoaging, and the reduction of the free radical injury in the epidermis is the most important strategy for a modern skin care product.

An antioxidant is defined as a molecule, belonging to a very heterogeneous group of molecules, able to reduce the amount of free radicals and to interrupt free radical chain reactions. This easy-looking process can work in consequence of very different and complex mechanisms and is influenced by several parameters.

(a) Free radicals are very reactive species with half-life times of nanoseconds or milliseconds. Therefore, an antioxidant has to react very quickly with free radicals in order to neutralize them.

(b) Most of the antioxidant classes neutralize free radicals by chemical reduction. Sometimes they become semistable radicals themselves, as in the case of tocopherol, ascorbic acid, or quinones. These radical intermediates must be neutralized as well, otherwise a chain reaction can occur that can also enhance the free radical injury.

(c) The oxidation state of an antioxidant determines its reduction potential. This chemical potential must be high enough to reduce free radicals. Especially flavonoids may present different oxidation grades due to fermentation processes that can significantly lower their antioxidative power.

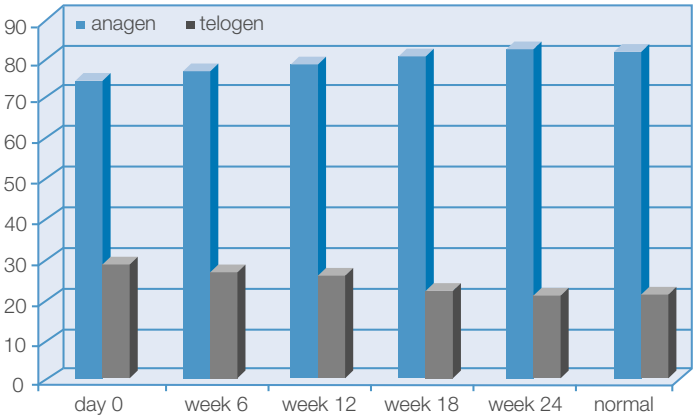
(d) The lipophilic or hydrophilic character of antioxidants determines the distribution inside the skin tissues and makes them more or less accessible to free radicals. Tocopherol, for instance, is able to interrupt radical reactions inside membranes, because of its lipophilic character.

(e) Several components of a cosmetic formulation are able to interact with active ingredients. It is important to determine the activity of antioxidants and their long term stability in the final formulations.

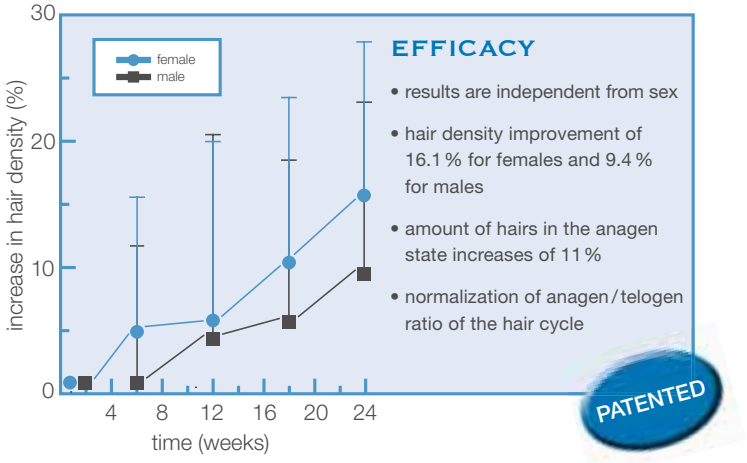
The need to consider all these influencing parameters reflects the complexity of antioxidant reactions.

ROVISOME Hair Growth Serum

ROVISOME Hair Growth Serum is an effective combination of the vitamins H (biotin), F (linoleic acid), E (tocopherol acetate), provitamin B5 (D-panthenol) and caffeine – significantly promoting hair growth and the normalisation of the hair cycle. In a human study carried out over a 6 month-period, a significant increase in hair growth of female and male volunteers applying 10 % ROVISOME Hair Growth Serum was observed. The hair quantity increased by 16.1 % per square centimeter. The hair cycle of women as well as of men was normalized in addition. An in vivo study on 12 tested persons clearly shows that within 24 weeks ROVISOME Hair Growth Serum shifts 11 % of hair from the telogen to the anagen phases.



The pecentage of hair bulbs in the anagen (growth) phase is 80 % under normal conditions. Lower percentage means hair loss. The results show a clear normalisation of the hair state.



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ROVI COSMETICS

There are different methods able to determine the antioxidative status of antioxidants, but it is very difficult to compare the results of different analytic methods. Some methods can not be applied for opaque or colored samples; other methods can not be applied for lipophilic substances and none of the most used methods quantify the reduction kinetics and the reaction velocity of the reaction between antioxidants and free radicals.

We want to present herein a method called Antioxidative Power (AP) able to quantify both the reaction capacity and velocity, that can be applied to all kind of samples, independent on their physicochemical properties, and that is able to compare different antioxidants by using the benchmark vitamin C. The AP method, based on ESR spectroscopy, provides the opportunity to obtain a meaningful parameter that characterizes the antioxidant system or mixture inside a cosmetic formulation. It can be used for the screening of different antioxidant classes and to compare the activity of raw material during the development process. Moreover, it is an excellent tool for the quality control of both raw materials and final products (2).

In the following the AP method is presented and some of the most commonly used antioxidants in modern skin care products are analysed and the different classes of antioxidants are compared and discussed.

■ Method

The measurements of the antioxidant capacity and reactivity of the different antioxidants were performed by using Electron Spin Resonance (ESR) spectroscopy. This technique is able to quantitatively detect free radicals and to analyse opaque, colored and viscous samples as well. The test radical 2,2-diphenyl-1-picryl-hydrazil (DPPH) was used in this assay. DPPH reacts with antioxidants and its signal intensity decay is recorded during the whole reaction. Different concentrations (at least 3 concentrations) of the test sample antioxidant were prepared and mixed with DPPH to obtain an initial radical concentration of 0.1 mM. The signal intensity decay of each concentration run is recorded at different time intervals (at least 7 times) during the reaction. From these intensities a first order kinetic is obtained for each concentration set. The kinetic parameters are used to calculate the reaction time t_r, that is a measure of the reactivity of the particular antioxidant under study. The static parameters of the first order kinetics are used to calculate the characteristic weight w_c, which reflects the capacity of the antioxidant. Both parameters are used to calculate the AP by means of the following equation:

$$AP(t,r) = \frac{RA \bullet N(DPPH-spin)}{t_r \bullet w_c} = \frac{RA \bullet c(DPPH)}{t_r \bullet w_c} \qquad (1)$$

where **N** is the quantity of reduced free radicals characterized by free electrons (spins) or the quantity of applied concentration **c** of DPPH-spins, **RA** is the reduction amplitude, w_c the characteristic net weight of the antioxidant, and t_r the corresponding reaction time.

For a better handling and the possibility of a direct comparison of the evaluated antioxidant activity of different products the AP has to be standardized to the activity of the established antioxidant substance ascorbic acid, supplied by Sigma Germany. The AP is based on the antioxidant activity of one milliliter of a solution with a concentration of 1 ppm vitamin C (ascorbic acid). This basic antioxidant activity is defined as an antioxidative unit (AU). Therefore, the AP of a solution containing vitamin C in a concentration of 1 ppm corresponds to 1 AU by the following relation:

$$AP\ (1\ ppm\ vitamin\ C) = 1\ AU = 2.495 \bullet 10^{13}\ spins/mg \bullet min. \qquad (2)$$

Accordingly the AP of vitamin C corresponds to an AP (vitamin C) = 10^6 AU = 2.495 10^{19} spins/mg min.

This standardization enables to create a benchmarked and reproducible measurement and allows to comparate directly very different classes of antioxidants.

■ Results and Discussion

Herein we present the AP values and reaction times of some antioxidants important for cosmetic formulations. These antioxidants belong to the class of vitamins (i.e ascorbic acid or tocopherols and their derivatives), and to the class of polyphenols. Some products contain also terpenes, carotenoids or other functional classes that may contribute to the antioxidant power. Some of the products are powders, others are oily formulations. Both lipophilic and hydrophilic substances are analysed. In **Table 1** and **Fig. 1** and **2** the AP values are reported together with the reaction times tr. These reaction times are included into the calculation for the AP values, but a separate consideration helps to understand the redox-mechanism of the antioxidants. Vitamin C and vitamin E have typical reaction times of 0.24 minutes and 0.33 minutes respectively. The derivates of these vitamins have considerably longer reaction times and are therefore less active than the parent vitamins. Tocopherol acetate has no *in vitro* antioxidant capacity, since the acetate has to be splitted off by esterase activity to reform the active tocopherol. Among the polyphenols aspalathin shows the highest antioxidative power, even higher than vitamin C. Also ellagic acid and rosmarinic acid show very high antioxidant activity. These polyphenols have also the shortest reaction times among the polyphenols under study. The shorter the reaction time is, the lower is the oxidation state of polyphenols and the higher is their antioxidative power. This consideration is important when herb extracts are analysed: during the production process of herbe extracts, the leaves or plants may undergo fermentation. This fermen-

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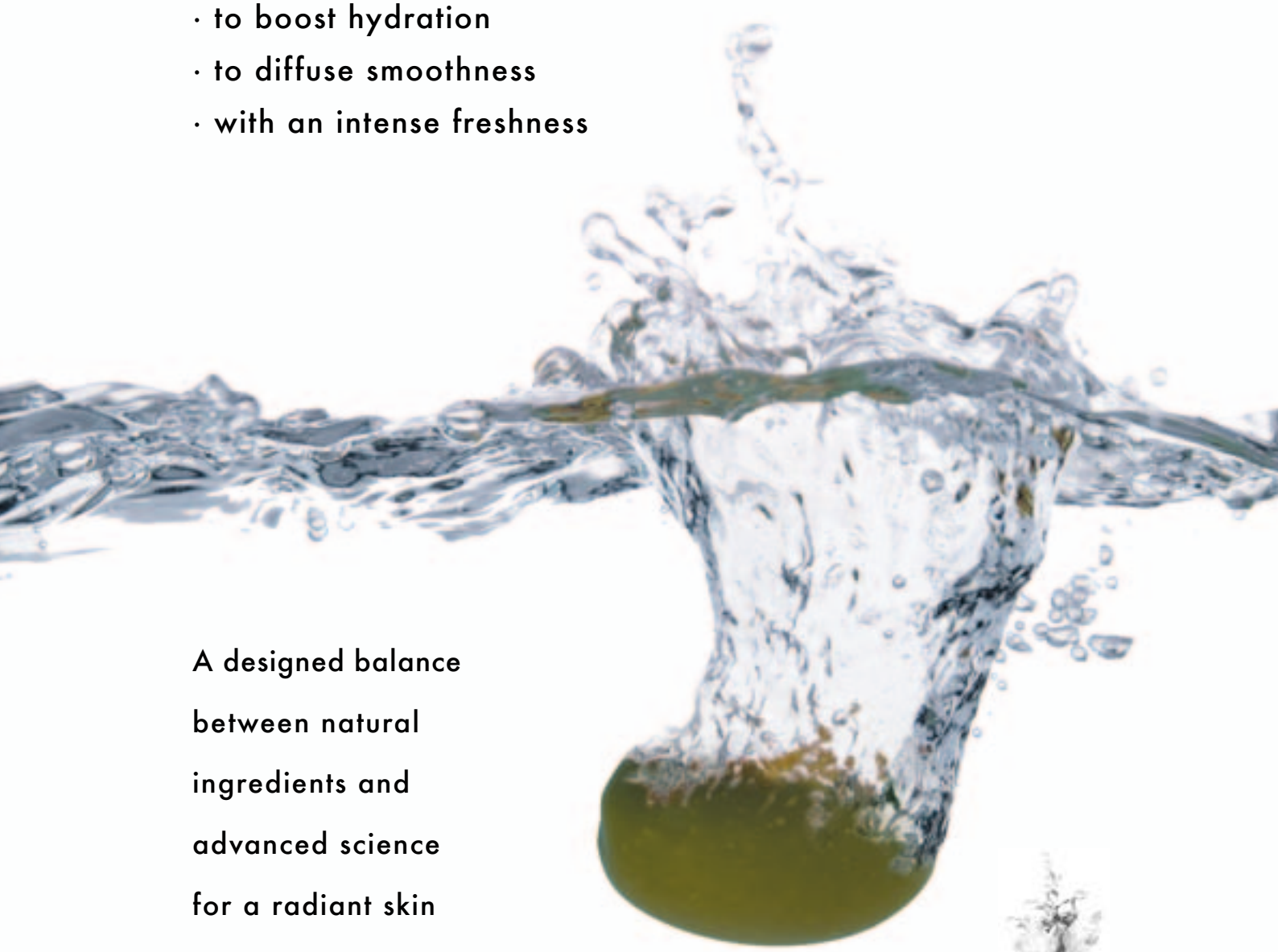

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tation leads to an oxidation of the polyphenols with consequently higher reaction times and lower AP values. Green tea leaves, for instant, have reaction times of about 0.35 minutes, whereas black tea – the fermented green tea – has reaction times of about 0.75 minutes (data not shown). The same consideration should be applied when Rooibos or Honeybush extracts are analysed. Five different Rooibos extracts from different suppliers showed AP values ranging from 90.000 AU up to 715.000 AU. Also the reaction times varied and fermented samples showed longer reaction times than unfermented ones. Also for grape seed extracts the AP values show a huge variability, depending on the production process and the oxidation of the active polyphenols.

Among the tocopherol and tocotrienol containing extracts we analysed amaranth seed oil, wheat bran extract and tocopherol liquid gained from sunflower seeds. Only the reduction kinetics of amaranth seed oil showed the typical reaction time of tocopherol. Wheat bran extract showed a very high reaction time of 2.87 minutes, that is typical for carotenoids.

In conclusion, from the above results appear that it is important to analyse carefully the antioxidant activity of bioingredients in order to produce an efficient cosmetic product. Many influences can have adverse effects on the antioxidant activity, such as fermentation, oxidation, and reactions of the active with the components of the cosmetic formulation. The AP method can be an excellent

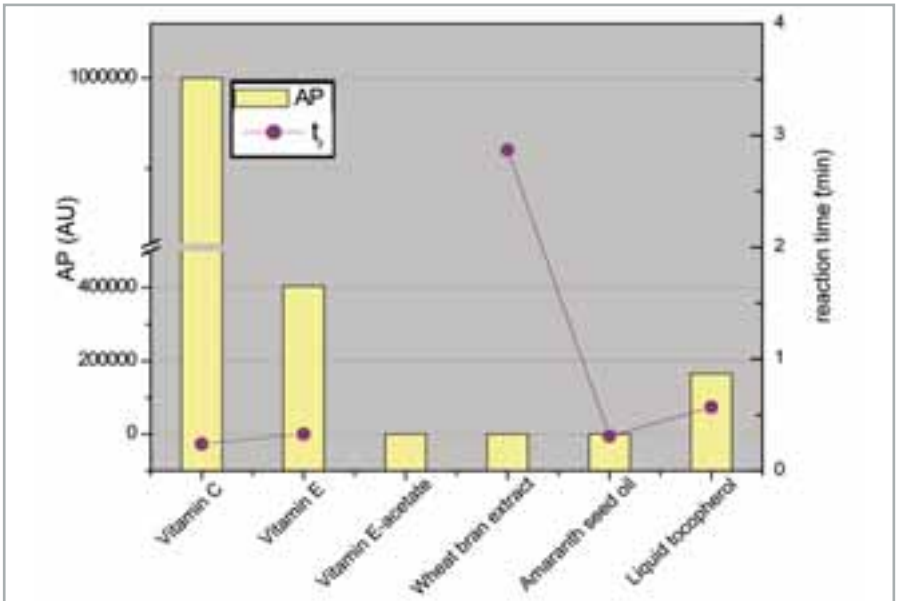

Fig. 1 AP and t_r of ascorbic acid, tocopherol and tocopherol derivatives

	Substance	AP (AU)	t_r (min)	Class	Form
1.	Vitamin C	1.000.000	0,24	Ascorbic acid	powder
2.	Vitamin E	404.000	0,33	tocopherol	oil
3.	Vitamin E-acetate	0		Tocopherol-acetate	powder
4.	Wheat bran extract	120	2,87	Tocopherols, carotenes	oil
5.	Amaranth seed oil	730	0,31	Tocopherols, tocotrienols	oil
6.	Liquid tocopherol	165.300	0,57	Tocopherols, tocotrienols	oil
7.	Ellagic acid	1.352.000	0,60	polyphenol	powder
8.	Rosmarinic Acid	971.200	0,51	polyphenol	powder
9.	Rosemary extract	243.500	0,79	polyphenol	powder
10.	Rooibos 1	90.000 –	0,331 –	polyphenol	powder
	Rooibos 2	715.000	0,79		powder
11.	Honeybush Extract	102.900	0,97	polyphenol	powder
12.	Aspalathin	1.531.000	0,22	polyphenol	powder
13.	Grape seed extract 1	357.000	0,95	polyphenol	powder
	Grape seed extract 2	930.000	0,81		powder
14.	Dihydroquercitin	1.030.000	0,23	polyphenol	powder
15.	Ginger Extract	98.700	0,73	terpene, polyphenol	oil

Table 1 AP and t_r values of the tested antioxidants

tool for the creation of a meaningful parameter of the antioxidant capacity and reactivity. First the raw materials and their long time stability should be tested in order to select a powerful antioxidant with the desired characteristics. Then, the final formulation containing the selected antioxidant should be tested again in order to exclude oxidation reactions. The active ingredients can be protected by adeguate procedures, for instance by encapsulation, to protect the antioxidant. To be effective, an antioxidant should be able to penetrate into the outer skin or hair which could be achieved by encapsulation strategies as well and should finally be able to neutralize free radicals. These efficacy tests can be performed using the SAP (Skin Antioxidative Potential) method on skin biopsies and/or the RHF (radical hair Protection Factor) on human hair (3,4). Both techniques use ESR spectroscopy and are suitable to demonstrate the antioxidant activities in skin and hair.

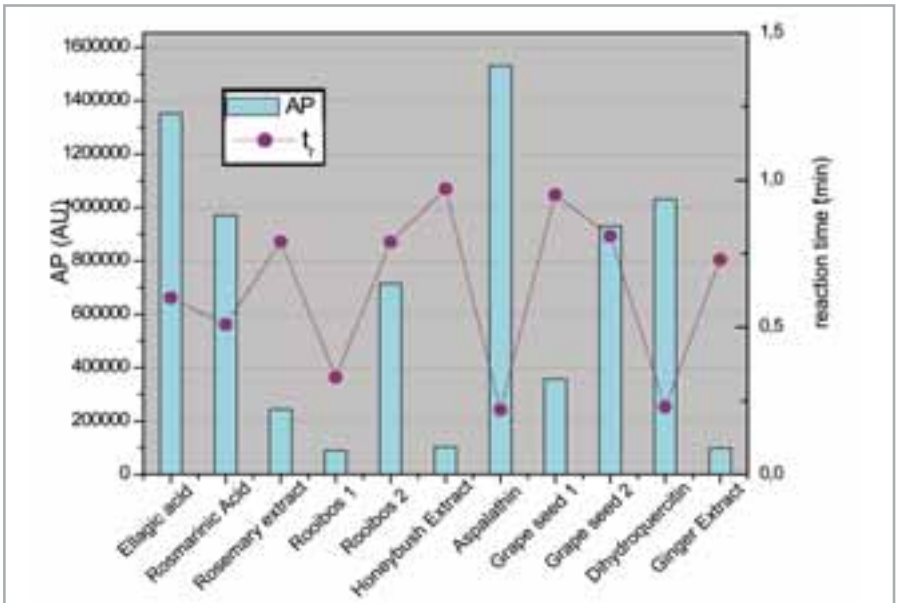

Fig. 2 AP and t_r of antioxidants of the polyphenol class

Bibliography

(1) DSM study. in: cosmetic web Business section, 2006

(2) *Jung K, Richter J, Kabrodt K, Lucke IM, Schellenberg I, Herrling T* (2006). The antioxidative power AP--A new quantitative time dependent (2D) parameter for the determination of the antioxidant capacity and reactivity of different plants. Spectrochim Acta A Mol Biomol Spectrosc 63:846-50

(3) *Jung K, Sacher M, Blume G, Herrling Th.* Anti-Aging Status of Skin characterized by the skin antioxidative Protection SAP – efficacy of topically applied antioxidants.. SÖFW 132, 9-2006, 38-44

(4) *Jung K, Herrling T, Blume G, Sacher M, Teichmüller D.* Detection of UV induced free radicals in hair and their prevention by hair care products. SÖFW 132, 7-2006, 32-36

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