Fragrance with Antiaging Benefits

Naturals-driven functionality for added value

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Natural fragrance companies are often interested in the olfactory impact that plant extracts can have on the design of new facets of fragrances. The hydrodistillation of plants yields essential oils characterized by molecules with relatively high volatility that often are used for the top and middle notes—the two most volatile fractions—of a fragrance. Traditional aromatherapy has claimed that essential oils have activity on the skin but only recently has research substantiated that essential oils can act as actives in cosmetic products. For example, the most recognized use of essential oils has been for antimicrobial activity against *P. acnes* or *M. furfur* for acne and antidandruff applications.[1,2] Moreover, studies have highlighted the use of essential oils as potential anti-inflammatory and antioxidant agents.[3,4]

To measure the antioxidant ability of essential oils, experiments must be carefully designed, as demonstrated by the work of Zhao and by the author, described here, since the design obviously affects the outcome.[5,6] For example, whereas some work has reported on the antioxidant activity of essential oils, work by the author has shown that only essential oils having a high quantity of phenolic molecules, such as eugenol and thymol, can act as efficiently as well-established free radical scavengers such as BHT and vitamin E.

Extraction from plants by volatile solvents yields an absolute that captures not only volatile notes, but also molecules with a sustainable odor, which contribute to the heart and bottom note of a fragrance. Quantitative analysis of most absolutes has identified heavier molecules such as flavons, diterpenes and triterpenoïds; esters and lactones; and polyols, acids, fatty acids and sterols that may have interesting cosmetic properties.[7–9]

One objective of the Robertet Aromacosmetic Laboratory is to study the different properties of these extracts and use them as building blocks for the development of functional active fragrances. Recent research has expanded to evaluate the effects of such materials on skin lightening, soothing and slimming. The aim of the present work was to investigate the complementary use of natural extracts in an active fragrance with broad spectrum activity against well-recognized skin aging factors. Previous in vitro tests performed at the company on single natural extracts were used to help perfumers create a fragrance containing more than 50% of several identified active compounds identified. Results reported here are obtained for a single active fragrance with multiple antiaging activities.[a]

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Skin Aging

To examine the activity of the tested extracts, one must first consider the mechanisms of skin aging factors. Skin aging is a complex phenomenon. The two most commonly recognized processes of aging are chronological, involving genetic factors, and photoaging, involving environmental factors. It is generally accepted that these mechanisms impact the physiology of skin by changing the evolution of extracellular matrix (ECM) proteins in proportion and organization via a degradation process initiated by matrix metalloproteinase, hyaluronidase and elastase. This process may be the key factor in the development of wrinkles.[10]

In the case of photoaging, exposure of skin to sunlight produces reactive oxygen species (ROS), free radicals and singlet oxygen. These damaging species have deleterious effects on constituents of the skin ranging from the sebum to the dermis, and are involved in the enzymatic cascade. To stop them, it is necessary not only to act against free radical species, but also singlet oxygen.

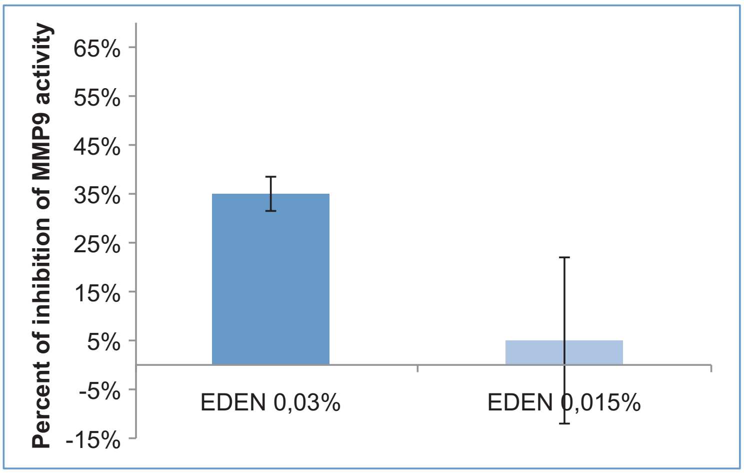
ROS: ROS species including singlet oxygen and free radicals react in different damaging ways on the skin. Free radicals promote the cycle of peroxidation via hydrogen abstraction, while singlet oxygen reacts following a Diels Alder or *ene* reaction to result in peroxide formation.[11] Thus, ROS play a complementary role in the auto-oxidation of lipids in the skin. To combat

[a] Actiscent Eden (INCI: Fragrance) is a product and registered trademark of Robertet.

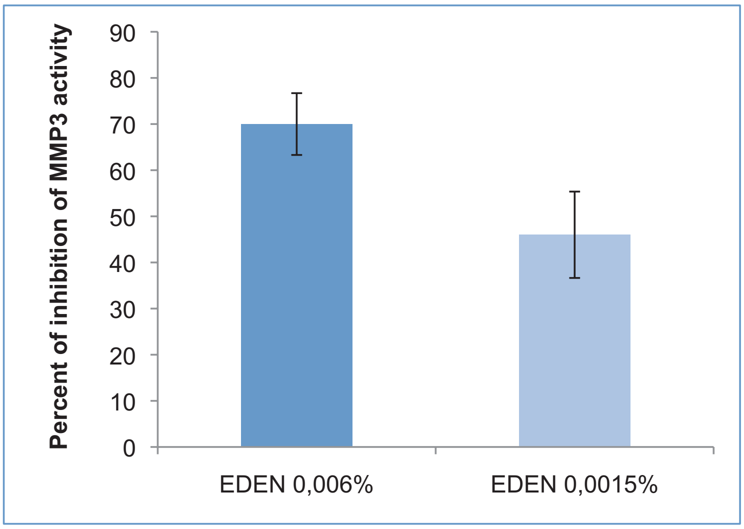
At a Glance

Fragrances are blends of synthetic chemicals, essential oils and solvent extracts, known as absolutes. Recent research has evaluated the effects of these natural aromatic products, revealing they may also positively impact processes in skin. Here, the author describes how a fragrance with specially selected naturals can provide pleasant scents as well as impart cosmetic activity.

Inhibition of human MMP-9 by the active fragrance **F-1**



Inhibition of human MMP-3 by the active fragrance **F-2**



the damaging effects of both species of ROS, multiple approaches are needed because free radical scavengers such as BHT alone are ineffective against singlet oxygen.[12]

Singlet oxygen has been shown to promote the creation of squalene monohydroperoxide in sebum and appears to be involved in the early formation of the primary radical in unsaturated lipids.[12,13] Consequently, squalene peroxide on guinea pig dorsal skin provokes erythema and histological changes such as acanthosis, hyperkeratosis and vacuole degeneration in the epidermis.[14] Moreover, squalene peroxide appears to be a major factor in skin wrinkle formation, as well as a comedogenic agent.[15,16] Studies on three-dimensional cultured human skin models suggests that squalene peroxide causes the peroxidation of cellular membrane lipids within the dermis and epidermis, and decreases the viability of keratinocytes and increasing interleukin-2 release.[17]

The role of free radicals in the formation of lipid hydroperoxide and in the depletion of natural antioxidants is well-documented.[18] The production of free radicals by UV exposure can even lead to the modification of the lipid bilayer and DNA. Moreover, free radicals appear to activate the production of matrix metalloproteinases (MMPs) through the activation of AP-1 transcription factor.[19]

Thus, preventing the start of the entire ROS process is a vital first step in defending against skin aging. The important second step would be to prevent the degradation of the ECM by the action of MMPs, elastase and hyaluronidase.

MMPs and serine proteinase: The family of MMPs is well-known for its role in degrading the ECM. This degradation is thought to be an influential factor in the initiation of skin wrinkles and skin sagging. The inhibition by the fragrance of two distinct metalloproteinases, MMP-3 and MMP-9 and a serine protease, the human leucocyte elastase, were studied in the present work since they are responsible for the degradation of different structural proteins.[20]

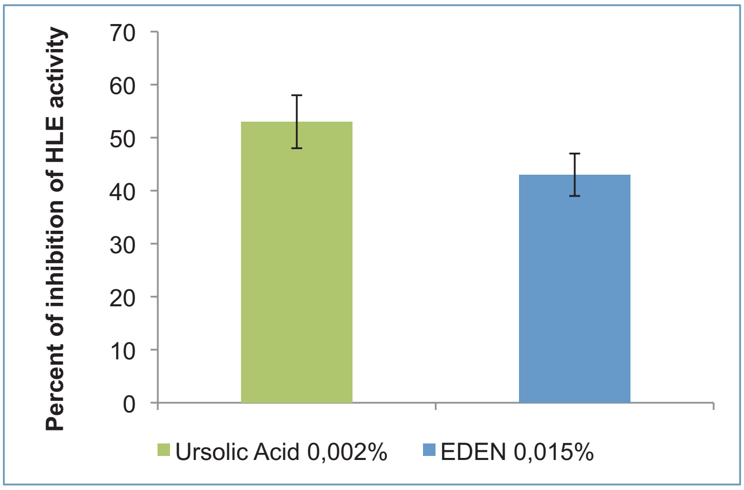
Human fibroblast stromelysin-1 or MMP-3 is a proteoglycanase with a wide range of substrate specificities. It is capable of degrading proteoglycan, fibronectin, laminin and type IV collagen. The process of secreting such metalloproteases is predominantly governed by connective tissue cells. MMP-3, upregulated in fibroblasts after UVB exposure, is an important contributor to the photoaging process.[21] The secretion of MMP-9, also known as 92-kD gelatinase or type V collagenase, is stimulated by UVB irradiation in a dose-dependent manner; it is actually decreased by UVA.[22] MMP-9 is also known to be an agent responsible for the photodegradation of skin collagen fibers.[20]

In addition, serine protease was examined. Skin exposure to UV promotes the activity of the serine protease known as human leucocyte elastase (HLE).[23,24] This enzyme acts as a mimic of true collagenase because it is responsible for the degradation of ECM proteins such as elastin, fibronectin and collagen, resulting in the loss of skin elasticity and the apparition of wrinkles.

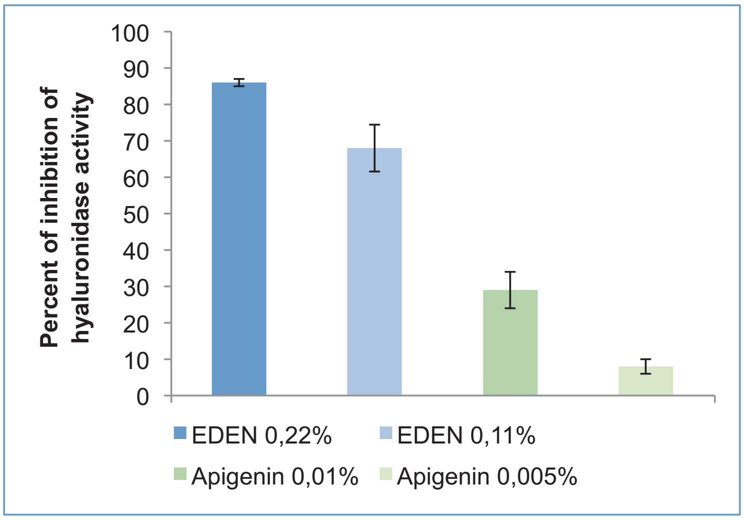
The inhibition of these enzymes by the fragrance would protect the main proteins of skin structure. Moreover, as the ECM content in glycosaminoglycans such as hyaluronan is an important part of the dermis structure, the inhibition of the hyaluronidase by the active fragrance also was studied.

Hyaluronan and hylauronidase: Hyaluronan is a high molecular weight polymer of repeating units of N-acetyl-glucosamine D-glucuronic acid that is synthesized in the plasma membrane of cells and translocated to the peri-cellular space. It is believed to have numerous important biologic functions, including modulation of cell proliferation, migration and differentiation, as well as the regulation of extracellular water and protein homeostasis. Produced by fibroblasts and keratinocytes, it is included in the ECM component of the skin.[25] Several studies have revealed the presence of hyaluronan in the dermis and epidermis, and indicate that it can be transferred in the stratum corneum (SC).[26] The degradation of this component is assumed to be linked with free radicals and hyaluronidase, which is regulated via UVB exposure but by a different pathway.[27,28]

Inhibition of HLE by the active fragrance **F-3**



Inhibition of hyaluronidase by the active fragrance **F-4**



Methods

To determine the antiaging benefits of the active fragrance in question, the author compared the active fragrance with two standard fragrances from the company's library and tested them at the same concentration in various in vitro and ex vivo protocols, described below. The increased activity of the active fragrance was due to its design around ingredients identified in previous studies as exhibiting efficacy against the skin aging factors described above. The active fragrance contained more than 50% of the active compounds discovered by the initial screening step. The control fragrances showed little or no activity on each of the protocols. Depending on the test, some ingredients of the active fragrance were removed since they caused interactions such as unusual coloration with the reagents independent of the activity of the enzyme studied, especially during the hyaluronidase testing. All testing was performed in triplicate.

MMP-3 and MMP-9: The ability of the active fragrance to inhibit the human MMP-3 and MMP-9 was evaluated by a colorimetric assay test method. The assay principle is based on the measurement of the degradation by the two enzymes of a chromogenic substrate (Ac-PLG-[2-mercapto-4-methyl-pentanoyl]-LG-OC2H5) (**see F-1 and F-2**).

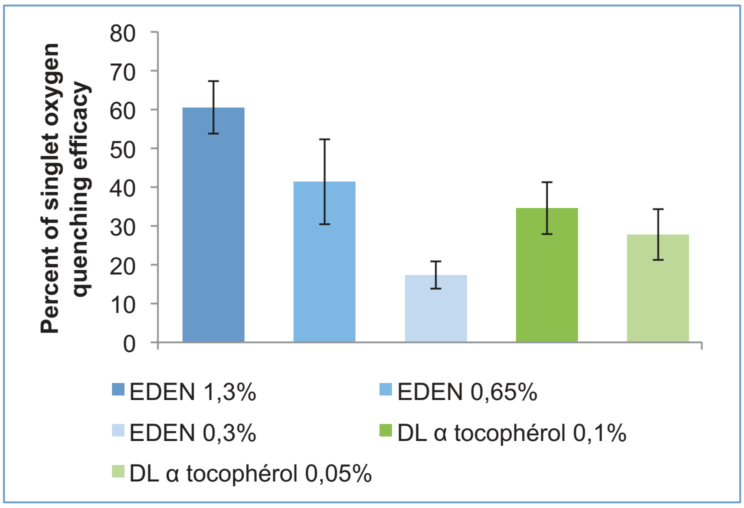
Elastase: The determination of the effect of the active fragrance on leucocyte elastase activity was performed according to Mitaine-Offer.[29] HLE activity was measured using N-(Methoxysuccinyl)-Ala-Ala-Pro-Val 4 nitroanilide as a substrate. The release of the p-nitroanilide subsequent to hydrolysis of the substrate was monitored by a spectrophotometer at 405 nm. A 0.015% concentration of the active fragrance was found to decrease the activity of the elastase by nearly 50% (**see F-3**).

Hyaluronidase: The determination of the effect of the active fragrance on the activity of hyaluronidase was measured following a modified colorimetric assay based on the reaction of N-acetyl-D-glucosamine at the reducing ends of hyaluronan and its fragments with p-dimethyl aminobenzaldehyde. This modification results in a red-colored product that can be monitored via a spectrophotometer at 590 nm.[30] The efficacy of the fragrance was compared with apigenin, and the active fragrance at 0.2% decreased the activity of hyaluronidase by 80% (**see F-4**).

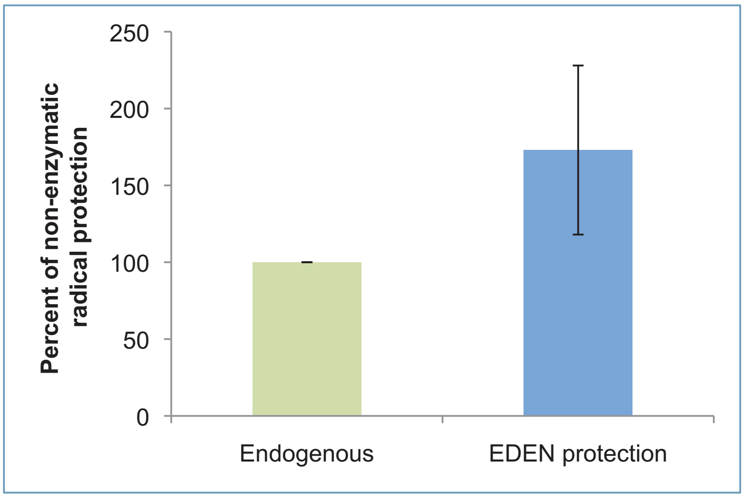
Anti-ROS Activity

Singlet oxygen: Singlet oxygen can be produced by photosensitization of Rose Bengal with an appropriate

Quenching of singlet oxygen by the active fragrance **F-5**



Measurement of the increasing antioxidant ability of skin extract 1.5 h after application of a cream containing 0.6% active fragrance **F-6**



wavelength of light. This sensitizer is excited and then will transfer its energy to triplet oxygen. The probe 1,3-diphenyl isobenzofuran, which undergoes direct reaction with singlet oxygen resulting in a loss of absorbance at 410 nm, can be monitored with a spectrophotometer.[31] The quenching activity of the fragrance on singlet oxygen can be monitored by comparing the loss of absorbance of the placebo versus the fragrance tested. From these results the researcher concluded that the active fragrance could be an efficient quencher of singlet oxygen (**see F-5**).

Ex vivo DPPH test: An ex vivo DPPH test method was used to measure the efficacy of the active fragrance on improving the free radical scavenging ability of the SC. Application on both forearms of a classical o/w skin cream containing the active fragrance at 0.6% and a placebo, and an o/w cream with a standard fragrance at 0.6%, was conducted by seven volunteers. All were asked to use the same amount of cream as they typically would for body applications. After application and a 1.5-h resting period, an extraction using ethanol on both forearms was made at three different sites and pooled together. These samples were filtered, and 0.8 mL of each was added to a precise amount of a diphenyl-2-picryl-hydrazyl (DPPH) solution at 50 mg/L. DPPH is a stabilized free radical that is used to predict the antioxidant activity of an extract; in this case, the ethanolic extract of the skin. The comparison is made with the placebo extract, which contains nonenzymatic antioxidant from the skin, extracted by ethanol, which is mainly vitamin E (α- and γ-tocopherol), considered in this test as the endogenous protection.[32] The results obtained showed an increase of the free radical scavenging ability of the ethanolic extract of skin 1.5 h after the application of the cream containing the active fragrance, compared with a standard fragrance (**see F-6**).

Conclusions

In the present study, a single active fragrance was tested in vitro and found to exhibit efficacy against five primary factors of skin aging. In addition, an ex vivo test revealed its free radical scavenging ability. The active fragrances developed were designed with complementary ingredients that have olfactive and cosmetic benefits to provide a broad spectrum activity. This active fragrance fulfills two important features of a cosmetic product; i.e., the pleasure of scent and antiaging activity.

From a formulation point of view, the advantages are numerous since the incorporation of fragrance is typically a trivial step in the formulation process, contrary to the inclusion of usual actives. Fragrance could thus become, more than ever, a crucial point in the formulation of a cosmetic product. Indeed, it could be of interest to formulators to add a fragrance with anti-hyaluronidase activity in a moisturizing cream containing hyaluronan, or to boost the antioxidant portion of a daily cream containing superoxide dismutase. Fragrances containing active natural ingredients demonstrate advantages in many cosmetic areas that are potentially beneficial for the skin.

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