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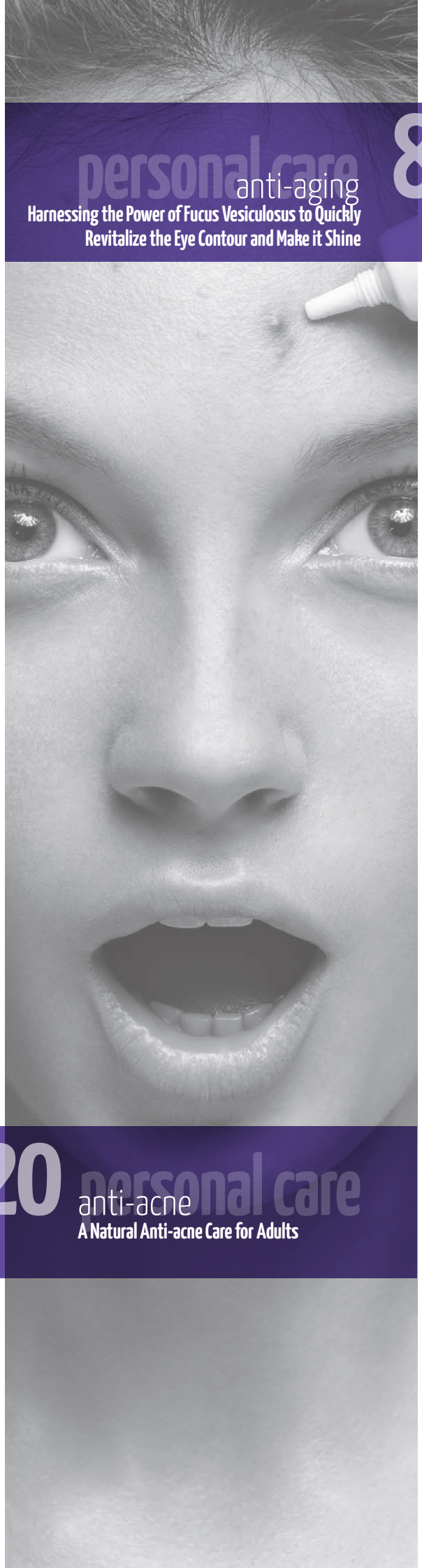

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Astaxanthin - the Diamond of Antioxidants in the Fight Against Light-induced Oxidative Stress

K. Dokulil, A. Pototschnik

abstract

The skin, as the largest organ of the body, plays an important role for our health. As a protective shield, it is constantly exposed to external environmental influences and stress. In order to maintain and support this protective barrier, numerous active ingredients are used in cosmetics. A major group of these are antioxidants, which can neutralize stress factors in the skin.
Astaxanthin, which is the strongest natural antioxidant belonging to the carotenoid family, does this particularly well due to its special molecular structure. Due to its unique structure of hydrophilic and lipophilic components, it can act more effectively in the lipid bilayers of the skin than most other antioxidants.
Especially in the field of light-induced oxidative stress, triggered by UV radiation and infrared as well as blue light, *in vitro* and *in vivo* studies with the active ingredient AstaCos® OL50 from BDI-BioLife Science have shown that astaxanthin has a strong protective effect.
Numerous research studies conclude that astaxanthin can also improve overall skin health, and provide comprehensive protection against oxidative stress. Possible uses range from anti-aging products to formulations that combat the effects of UV exposure and light, to an active ingredient in sunscreen products and face creams.

Introduction

As the largest organ of our body, the skin plays an important role as a protective barrier. Its health and morphology determine our appearance and contribute significantly to our well-being.

As a protective shield, the skin is constantly exposed to external environmental influences and stress caused by internal stress factors such as athletic exertion and external stress factors such as UV rays. In order to maintain and support the skin as a protective barrier, numerous active ingredients are used in cosmetics. Antioxidants form a large group of these; they can neutralize stress factors in the skin.

Astaxanthin is considered the strongest natural antioxidant and belongs to the carotenoid family. Due to its special molecular structure (carbon, hydrogen and oxygen atoms), it belongs specifically to the xanthophylls [1]. Basically, natural astaxanthin can be produced in a unique biochemical process by photosynthetic bacteria, algae and yeasts. The highest concentrations are accumulated by the microalga *Haematococcus pluvialis*. In the microalgae, the molecule astaxanthin is directly linked to phospholipids and is present as monoesters and diesters, depending on whether the fatty acids react with one hydroxyl group or with both hydroxyl groups [2]. As a cosmetic ingredient, it is gently extracted from *Haematococcus pluvialis* crude biomass by supercritical CO_2 extraction and is fat soluble.

Fig. 1 AstaCos® OL50, *Picture credits: Helmut Pierer*

The molecular structure of astaxanthin

Astaxanthin has 40 carbon atoms and with its 13 conjugated double bonds, it provides a tremendous antioxidant capacity to eliminate oxidative stress and free radicals. [3] Due to its unique molecular structure, it is highly effective against reactive oxygen species (ROS) without showing pro-oxidant side effects such as vitamin E, lycopene or ß-carotene. In direct comparison, astaxanthin appears 6000 times more potent than vitamin C and 100 times more potent than vitamin E in neutralizing singlet oxygen [4]. Its naturalness, enormous efficiency and uniqueness make it the "diamond of radical scavengers".

As an antioxidant, astaxanthin can be used in a wide variety of cosmetic applications. It has both hydrophilic and lipophilic components in its structure. It can therefore act more effectively in the lipid bilayers of the skin than most other antioxidants. As a fat-soluble ingredient, it opens up numerous processing possibilities in cosmetics, and its natural vegan red color gives cosmetic applications a unique selling point **(Figure 2)**. AstaCos® OL50 is a cosmetic active ingredient developed by BDI-BioLife Science GmbH. The active ingredient with 5% astaxanthin dissolved in high-quality jojoba oil was awarded COSMOS-CERTIFIED in 2021 **(Figure 1).** Possible applications range from anti-aging products to formulations, combating the effects of UV exposure and light.Furthermore AstaCos® OL50 can support as an ingredient in sunscreen products and face creams.

Fig. 2 Color spectrum of AstaCos® OL50 in an O/W emulsion

Light-induced oxidative stress and the role of ROS (reactive oxygen species).

Light-induced oxidative stress plays a crucial role in human skin aging and skin damage. Whenever skin molecules absorb UV light or visible light and transfer energy to oxygen, they enter an excited state and form singlet oxygen. Skin photosensitizers transfer charge to oxygen and cause ROS and free radicals. Photosensitizers are endogenous or exogenous compounds that are readily activated by UV or visible light and once activated, cause an adverse skin reaction. This can result in the formation of ROS, highly reactive chemical molecules formed due to the electron accepting capacity of oxygen. The light-induced ROS and free radicals lead to oxidative stress, wreak havoc on proteins, lipids and DNA, and cause negative changes in skin structure. Singlet oxygen is an extraordinarily reactive molecule and its impact on cellular DNA is particularly disastrous for skin.

When singlet oxygen attacks cell membranes, it activates enzymes that lead to cell death, peroxidation and ultimately deterioration of the skin's appearance. A singlet oxygen molecule can introduce a single 8-OH-dG (8-hydroxydesoxyguanosine - biomarker for oxidative stress) into a DNA molecule. This process triggers the so-called NFkB cascade - the beginning of inflammation, which results in a chain reaction of thousands of molecular modifications in the cell, membrane, and the overexpression of MMP-1 enzymes, which destroys collagen and other elastic fibers. Thus, skin aging is subsequently driven [1,5].

Light-induced oxidative stress & factors that can trigger it

Light-induced oxidative stress is triggered by various types of radiation, such as:

- UVA radiation
- UVB radiation
- infrared light
- blue light

UVA and UVAB radiation (ultraviolet light) and infrared light are responsible for approximately 50-80% of visual skin aging. Therefore, light protection is an essential feature of cosmetic approaches against premature skin aging. In particular, the amount of UVB in sunlight is a critical, well-characterized factor in skin damage. UVB radiation, a high-energy, short-wavelength radiation (290 - 320 nm), basically stresses the epidermal part of the skin. In extreme cases, this leads to severe sunburns and damage to the apical as well as proliferating basal keratinocytes [6].

In contrast, the UVA portion of sunlight is lower in energy and more long wavelength in nature (320-400 nm). This type of irradiation can penetrate the dermal parts of the skin, resulting in long-term damage to collagen and elastin fibers. Long-term and combined irradiation of human skin with UVB and UVA leads to a reduction and slowing down of the skin renewal potential as well as structural damage to the dermal matrix, This eventually results in permanent physical relaxation of the skin. Human skin itself possesses intrinsic cellular protective mechanisms capable of regulating the externally induced amount of ROS to a tolerable minimum. This natural protective mechanism can be overloaded by regular and continuous UV irradiation and weakens more and more over time. Sunscreen filters such as zinc oxide and titanium oxide can ward off some of this radiation, but not all of the radiation that affects our skin. Complementary ingredients such as antioxidants, especially astaxanthin, play a key role in cosmetic formulations to prevent premature photoaging of the skin and act as a supplement to sunscreen filters [7].

The positive properties of astaxanthin for protection against the effects of UV radiation were confirmed with the active ingredient AstaCos® OL50 in in vivo and *in vitro* studies.

In an *in vitro* study, keratinocytes exposed to intense UV-B radiation were compared. Keratinocytes were treated with a concentration of 0.05% to 0.1% AstaCos® OL50 prior to irradiation. Compared to the untreated, non-irradiated refer-

ence tissue (see **Figure 3** area (B)), severe tissue damage of all epidermal layers was detected. The untreated, irradiated models (B) show clear signs of excessive UV irradiation and sunburned cells (**Figure 3** area (B) yellow arrows) - these are indicated by constricted nuclei. In addition, hydropic cells/areas (**Figure 3** area (B) red arrows) with less intense staining can be identified, which are the cause of cytosolic fluid accumulation. In addition, many vacuolated keratinocytes were found in the epidermis, reflecting typical tissue destruction after strong UV exposure as well as incipient parakeratosis. Compared to the untreated irradiated skin models, the tissue treated with 0.05% AstaCos® OL50 showed a much milder UV-B phenotype without parakeratosis (**Figure 3** area (C)). The tissue was less vacuolated and contained fewer apoptotic cells and vacuolated keratinocytes. In addition, significantly fewer sunburned cells (C, yellow arrows) and apoptotic cells were found compared with the positive control. Overall, epidermis treated with the product left a healthier, more vital impression and surviving basal keratinocytes still exhibited the typical elongated morphology (compared to area (A) in **Figure 3**). This indicates that basal keratinocytes are largely unaffected by UV irradiation. The results of the described in vitro study confirm the statement that the product AstaCos® OL50 has a strong protective effect against UV-B radiation in the reconstructed 3D human skin models used.

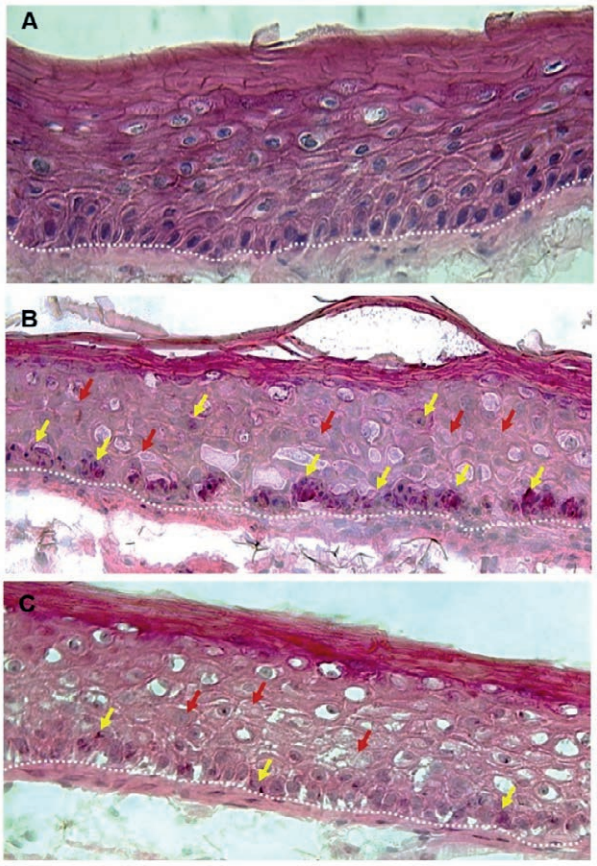

Fig. 3 Exemplary representation of UV-B irradiated 3D full skin models with H&E staining after 24 hours. A) Untreated control tissue, not irradiated (reference). B) Untreated tissue irradiated with 200 mJ/cm² (positive control). C) 0.05% tissue treated with AstaCos® OL 50 after irradiation with 200 mJ/cm². Yellow arrows: sunburned cells (SBCs), red arrows: hydropic cells or tissue, dashed white line: epidermal-dermal transition zone.

The protective effect against the effects of UV radiation of AstaCos® OL50 was also confirmed in an in vivo study. In a pilot clinical study, AstaCos® OL50 was used at a concentration of 0.2%. Its UV-protective effect compared to a placebo was tested in a controlled experimental setting on 21 healthy volunteers with Fitzpatrick skin type 2 or 3. After intensive UV exposure, a 25% lower erythema level was measured with the AstaCos® OL50 treatment compared to the placebo group. In this experimental setting, the active ingredient suppressed visible erythema formation in over 70% of cases [8].

Just like UVA and UVB radiation, blue light also has significant effects on our skin.

Blue light causes oxidative stress, especially in the mitochondria. The visible part of the light causes physiologically detectable changes in the skin, because blue light irradiation leads to a reduction of flavins. The ROS synthesis triggered by blue light probably does not react as singlet oxygen, but as superoxide. Results of numerous studies suggest that blue light has a similar effect on skin aging as UVA radiation [9].

In a recent study, UV-VIS spectroscopy revealed that AstaCos® OL50 has an absorption maximum of 476 NM.

Blue light is referred to a wavelength of 400-480 NM. Accordingly, AstaCos® OL50 is able to provide significant protection against blue light induced skin damage [10].

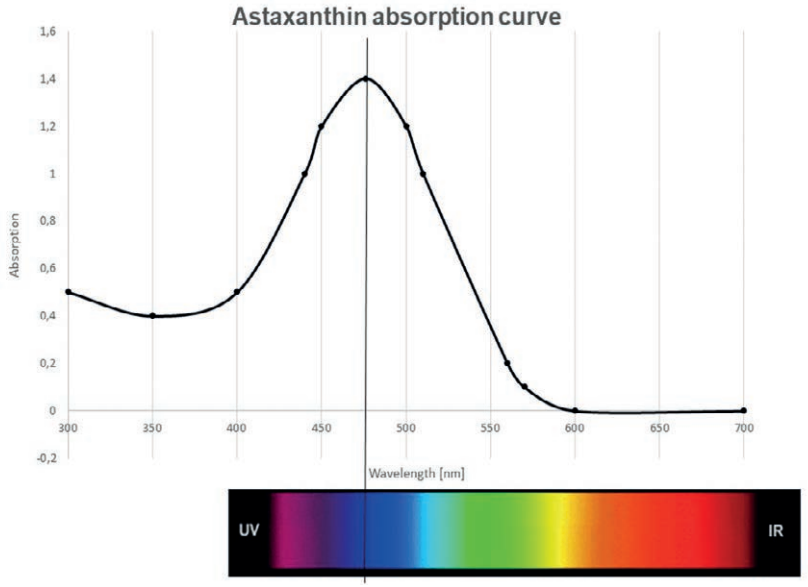

Fig. 4 UV-VIS spectrum of AstaCos® OL50

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Conclusion

Numerous research studies lead to the conclusion that astaxanthin can improve the overall health of the skin, providing comprehensive protection against oxidative stress. The active ingredient AstaCos® OL50 with astaxanthin, the diamond among free radical scavengers, acts exactly where it is needed due to its multiple properties and penetrates deep into the skin layers. In particular, the protection against the effects of radiation in the UVA and UVB and blue light range should be highlighted. Astaxanthin from microalgae is perfect for use in leave-on products. Due to its refinement as oleoresin, the power antioxidant can be ideally incorporated into a wide variety of formulations such as facial applications, pre-treatments and sun protection products and protects our skin against light-induced oxidative stress.

About BDI-BioLife Science

BDI-BioLife Science is a specialist in the development of innovative technologies for the production of high quality algae valuables for the life science industry.

In the production plant located at Ökopark Hartberg/Austria, BDI-BioLife Science produces high-quality natural astaxanthin tailor-made for the cosmetics (AstaCos®) and nutritional supplements (AstaFit®) industries using its in-house developed, closed algae cultivation process.

References:

[1] *S. Davinelli, M. E. Nielsen, and G. Scapagnini*, "Astaxanthin in skin health, repair, and disease: A comprehensive review," Nutrients, vol. 10, no. 4, pp. 1–12, 2018.

[2] *R. R. Ambati, P. S. Moi, S. Ravi, and R. G. Aswathanarayana*, "Astaxanthin: Sources, extraction, stability, biological activities and its commercial applications - A review," Mar. Drugs, vol. 12, no. 1, pp. 128–152, 2014.

[3] *H. D. Martin et al.*, "Chemistry of carotenoid oxidation and free radical reactions," Pure Appl. Chem., vol. 71, no. 12, pp. 2253–2262, 1999.

[4] *Y. Nishida, E. Yamashita, and W. Miki*, "Quenching Activities of Common Hydrophilic and Lipophilic Antioxidants against Singlet Oxygen Using Chemiluminescence Detection System," Carotenoid Sci., vol. 11, no. December, pp. 16–20, 2007.

[5] *K. Scharffetter-Kochanek et al.*, "Photoaging of the skin from phenotype to mechanisms," Exp. Gerontol., vol. 35, no. 3, pp. 307–316, 2000.

[6] *D. R. Bickers and M. Athar*, "Oxidative stress in the pathogenesis of skin disease," J. Invest. Dermatol., vol. 126, no. 12, pp. 2565–2575, 2006.

[7] *D. G.-R. institute for reliable Results*, "Physiological / Histological in vitro Expertise," no. March, 2021.

[8] *P. D. Kopera*, "UV-protektives Potential von Astaxanthin Halbseiten-kontrollierte Pilotstudie," no. 32, pp. 1–14, 2021.

[9] *Y. Nakashima, S. Ohta, and A. M. Wolf*, Blue light-induced oxidative stress in live skin, vol. 108, no. March. 2017.

[10] "200610_Spektrum_Bluelight_Test_Derma1418.pdf." .

DI Katharina Dokulil

is Head of Product Development at BDI-BioLife Science. She studied biotechnology at the Graz University of Technology and played a key role in the development of the company's proprietary algae cultivation process. With her team she works on the optimization of algae-based raw materials, development of raw material innovations and customized product concepts.

Alexander Pototschnik, MA

is Product Development Manager at BDI-BioLife Science. He studied food product development and resource management at the University of Applied Sciences in Wiener Neustadt, Wieselburg, and gained professional experience as a production manager at a cosmetics company. At BDI-BioLife Science he works with the active ingredient AstaCos, develops it further and deals with its application in cosmetic formulations.

authors

Katharina Dokulil, Alexander Pototschnik

BDI-BioLife Science
Parkring 18 | 8074 Raaba-Grambach | Austria

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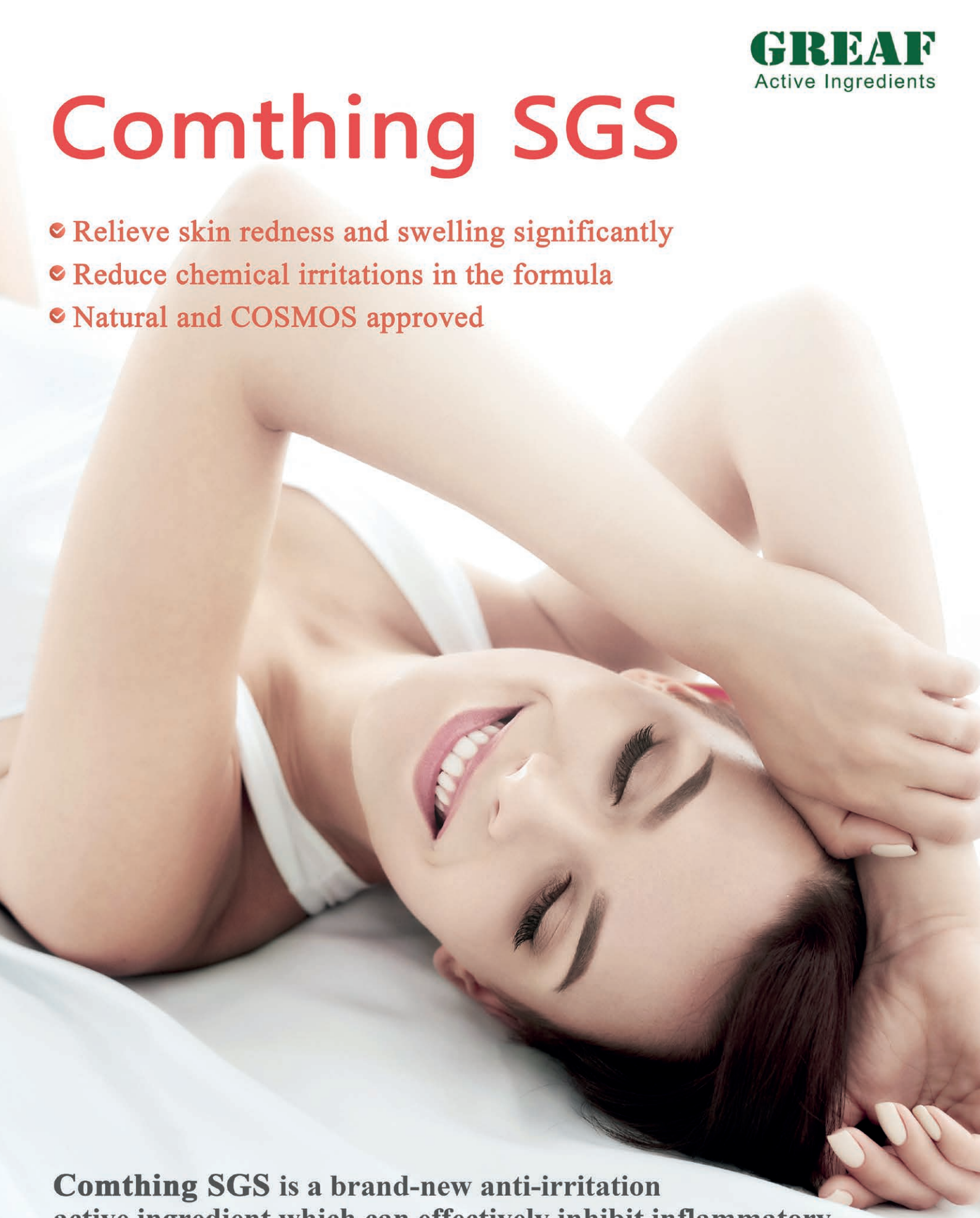

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Harnessing the Power of *Fucus Vesiculosus* to Quickly Revitalize the Eye Contour and Make it Shine

C. Reymermier, N. Pelletier, L. Danoux, W. Chan, C. Boury, V. André

abstract

The eye area is the first part of the body to reveal visible evidence of fatigue, stress, unhealthy lifestyle, and aging. Dark circles, crow's feet and dull skin are widely perceived to be telltale signs. As a result, many consumers are seeking safe and easy-to-use products that can protect their eye contours and limit these facial skin problems. Innovators at BASF have developed a new bioactive ingredient rich in fucoidan that is clinically proven to fight dark circles, reduce the appearance of wrinkles and enhance skin luminosity around the eyes within the first week of application. Its name is Seanactiv™*.

Introduction

The eye area is the most important part of the face, being our best asset to intensively communicate with others, but also being our emotional mirror. And in a modern digital daily life, with excessive use of computers and smartphones, but also with prolonged reading, short nights, and long working hours, we often forget to take care of this fragile part of our body. On top of this, unhealthy diet, stress but also genetics and ethnicity have an influence on the eye contour appearance. Recent research has shown that the perception of age, health and attractiveness is largely affected by several facial skin ageing features [1]. This includes dark circles, crow's feet, brown spots and the openness of the eyes [2,3]. In fact, dark circles and wrinkles are key elements in the assessment of perceived age or fatigue [4,5]. Consumers are highly aware of these perceptions, particularly because the eyes are often the most visible part of the face.

Demand is increasing for solutions that can combat these challenges and restore healthy-looking eyes. Skin conditions such as dark circles, fine lines and dehydration are high concerns especially for younger consumers, with almost 49% of UK 16 - 24 year olds who use facial skincare saying they have dark circles under the eyes. Also, in the US, 25% of women say that their eyes are their biggest concern when it comes to maintaining their healthy skin. Overall, as many as 96% of consumers worldwide have indicated that they want to reduce dark circles, while 66% want to decrease signs of aging [6].

In search of the perfect solution to improve their eye contour appearance, consumers are trying a wide range of approaches. This can include serums and creams, using concealer to cover dark circles, patting the skin around the eyes to enhance microcirculation, applying masks or using special tools. Some consumers even resort to more drastic measures such as lifting, Botox or blepharoplasty.

There is a clear need for solutions that can help to erase signs of aging around the eyes quickly and effectively. BASF has now launched Seanactiv™ (INCI: Water (and) Fucus Vesiculosus Extract (and) Gluconolactone (and) Xanthan gum (and) Sodium chloride), a new active ingredient that helps to erase signs of aging around the eyes quickly and effectively – with perceivable results within just one week of application.

A new algae active ingredient rich in fucoidan

Seanactiv from BASF is the latest innovation for anti-aging cosmetic products that target the eye contours. It uses the power of fucoidan within a well-known species of algae to quickly achieve visible results. *Fucus vesiculosus*, commonly known as bladderwrack, grows in the clear northern hemisphere waters of Nova Scotia and Brittany. Its reported health effects are largely attributed to non-digestible polysaccharides (dietary fiber) and polyphenols. Compared to other algae, *Fucus vesiculosus* contains the highest level of the polysaccharide fucoidan [7].

Fucoidan from algae is a sulphated polysaccharide characterized by fucose monomers. The algae used for Seanactiv is harvested manually from May to September under government license, in line with good collection practices that ensure regeneration of the biomass, and it is also certified as organic. It is extracted through a process that concentrates the active fucoidan molecule present in the algae.

By leveraging the power of fucoidan, Seanactiv has a quick and positive effect on diminishing both signs of eye fatigue and aging of the eye area. It offers a three-step action that

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has been proven by *in vitro* and *in vivo* tests. First, it contributes to reduce dark circles by stimulating the production of heme oxygenase. Second, it helps to reduce the appearance of wrinkles and achieve younger skin appearance thanks to its effect on the production of Collagen I. Finally, it contributes to an increase in the production of the Sirtuin-1 enzyme (SIRT-1) which consequently increases the mitochondrial activity and Adenosine triphosphate (ATP) production. It thus helps to improve overall skin luminosity of the eye area.

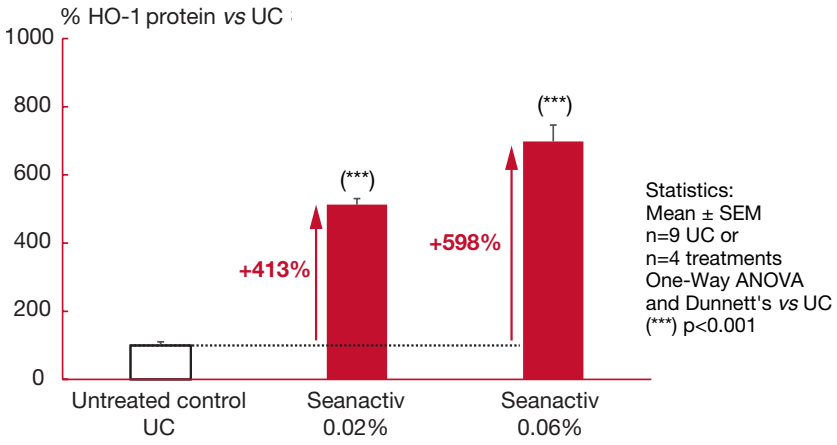

Fig. 1 Evaluation of the effect of Seanactiv on HO-1 synthesis by normal human epidermal keratinocytes.

Stimulating heme oxygenase synthesis to help reveal eye freshness

The first *in vitro* test evaluated the ability of Seanactiv to stimulate heme oxygenase-1 (HO-1), an enzyme that promotes heme degradation into less intensely colored products, which also show cell-protective effects. This helps to limit the appearance of dark circles around eyes – which is the top priority of consumers. Seanactiv was applied to cultured human keratinocytes ($p<0.001$) at 0.02% and 0.06%, using a capillary electrophoresis-based protein analysis system (Sally Sue) to quantify the presence of HO-1 protein.

Seanactiv stimulated synthesis of HO-1 protein by human keratinocytes by 413% and 598% at 0.02% and 0.06% respectively compared to the untreated control (UC) **(Figure 1)**. This shows its potential to break down the intensely colored heme and to contribute to limit the appearance of dark circles under the eyes. The degradation products of heme also support

cell protection. As demonstrated by similar results obtained with the standard fucoidan molecule (data not shown), the activity was supported by the ingredient's richness in fucoidan.

Stimulating Collagen I synthesis to help revive eye contour youthfulness

In vitro tests also investigated the capacity for Seanactiv to stimulate Collagen I synthesis in the extracellular medium in a model of cultured dermal fibroblasts, while evaluating whether the characterized molecule fucoidan was responsible for this effect. Wrinkles and fine lines are linked to loss of Collagen I and cell senescence. Stimulating Collagen I synthesis should help to counteract the decrease in collagen fibers in the skin, while helping to regenerate and strengthen the dermal extracellular matrix to limit the appearance of wrinkles. Normal human dermal fibroblasts were prepared and treated with Seanactiv at 0.02%, with a standard fucoidan molecule at dry weight (dw) or without any product (UC). This experiment used a method based on the DELFIA® method developed by BASF Beauty Care Solutions France SAS that allows very precise quantification of extracellular mature (or deposited) Collagen I.

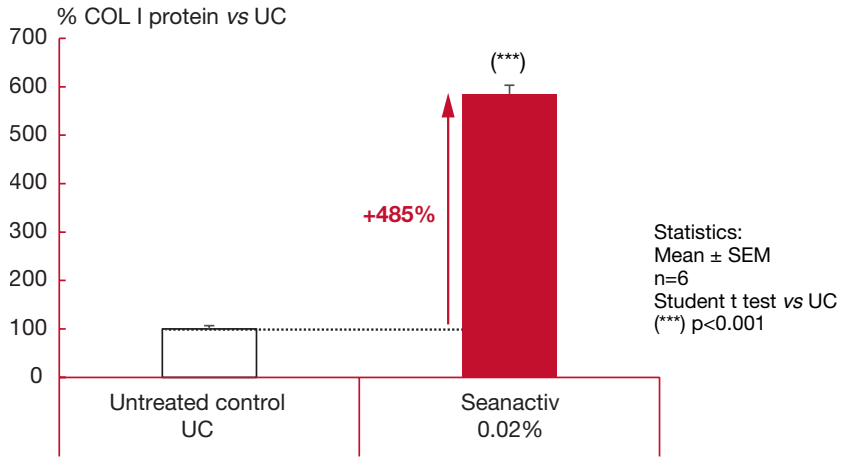

Fig. 2 Evaluation of the effect of Seanactiv on Collagen I synthesis by normal human dermal fibroblasts.

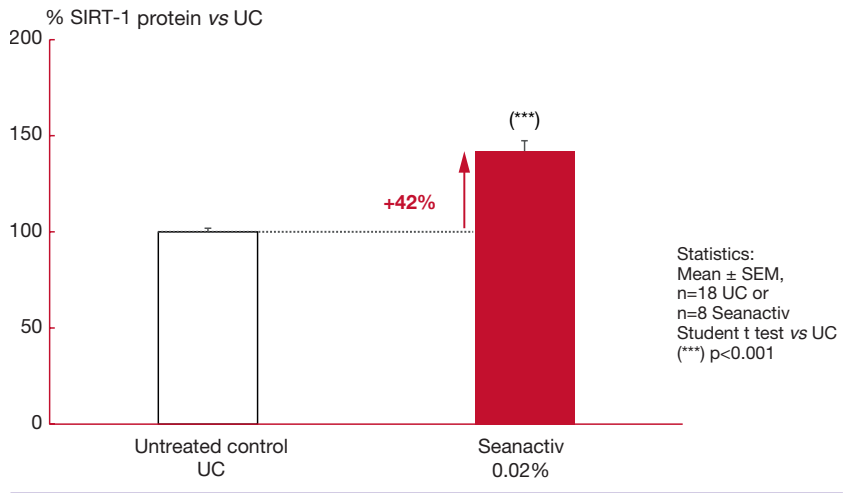

Fig. 3 Evaluation of the effect of Seanactiv on SIRT-1 production by normal human dermal fibroblasts.

Seanactiv stimulated the *in vitro* synthesis of Collagen I by 485% compared to the untreated control (UC) in cultured human fibroblasts ($p<0.001$) **(Figure 2)**. As demonstrated by similar results obtained with the standard fucoidan molecule (data not shown), the activity was supported by the ingredient's richness in fucoidan.

Stimulating SIRT-1 synthesis for cell vitality to help offer a wave of radiance

Further *in vitro* tests explored the capacity for Seanactiv to improve cell vitality and limit cell aging and senescence by reactivating the synthesis of epigenetic SIRT-1, as well as ATP production and mitochondrial activity. SIRT-1 enzyme is an epigenetic regulator and protector of cell longevity. It limits histone acetylation that is caused by aging or UV exposure, and is also associated with cell energy, longevity and mitochondrial activity. Synthesis of SIRT-1, production of ATP and mitochondrial activity are three indicators of aging phenotype that contribute to low cell activity or vitality, and cell longevity [8].

Boosting ATP production or mitochondrial activity could help to produce a more youthful, radiant and healthy appearance of the skin [9]. The experiment used a capillary electrophoresis-based protein analysis system (Sally Sue) that allows quantification of SIRT-1. ATP production was evaluated with luminescent assay and mitochondrial activity was investigated with spectrophotometry assay. The cells were treated with or without the active ingredient at a concentration of 0.02% for 24 hours.

Seanactiv *in vitro* stimulated the synthesis of SIRT-1 by 42% compared to UC in cultured human fibroblasts ($p<0.001$; **Figure 3**). As demonstrated by similar results obtained with the standard fucoidan molecule (data not shown), the activity was supported by the ingredient's richness in fucoidan. As a result of SIRT-1 stimulation, ATP production and mitochondrial activity were enhanced by 258% and 138% respectively compared to UC in cultured human fibroblasts (data not shown). These results indicate the potential for Seanactiv to limit cell aging and increase cell vitality in the dermis to help to improve the appearance of dull skin.

Improving the appearance of dark circles and crow's feet

In vivo tests have also been conducted to examine the capacity for Seanactiv to reduce the appearance of dark circles and wrinkles around the eyes. The study involved 24 healthy female volunteers aged between 18 and 65 who had crow's feet wrinkles grade 3-4 (assessed by a clinical scientist) and/or eyebags and/or undereye dark circles. A cream containing 1% Seanactiv was applied to the face once in the morning and once in the evening in a double-blind randomized, split-face and placebo-controlled study. The effect on the eye contour was evaluated during two periods of time – a short period (D1 to D7) and a long period (D1-D28). Measurements were conducted every day by volunteers at home after waking up, just before the morning application. Undereye images and crow's feet area images were assessed using a new device called SkinCam that can be connected to a smartphone. This allowed day-to-day image acquisition throughout the treatment, with the images quickly transferred to the expert analyzer. After a quality control check to eliminate low-quality images and data, 22 and 18 subjects were included in the final analysis for undereye area and crows' feet area respectively.

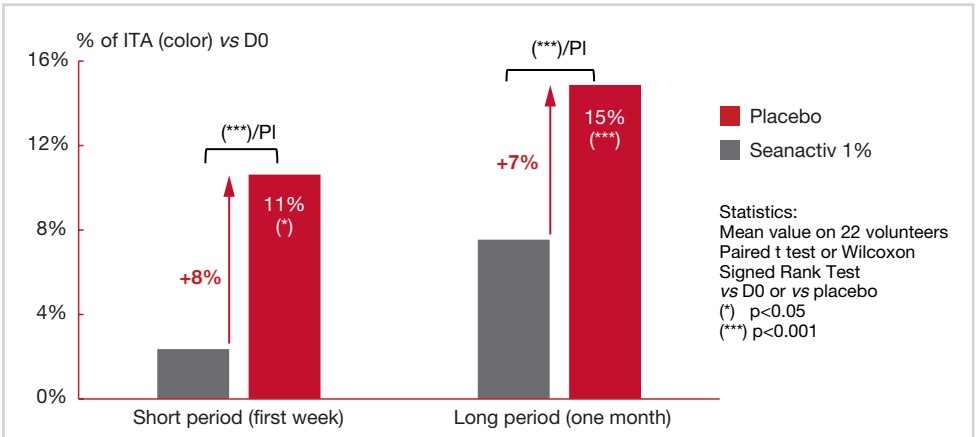

Fig. 4a Calculation of the percentage change of the ITA value vs baseline during two periods of time: a short one (first week) and a long one (one month). Day-to-day measurements conducted at home with skinCam connected device. Dark circle color measurement thanks to ITA value evaluation.

Seanactiv decreased the dark circle color by increasing the Individual Typology Angle (ITA) value by 11% during the short period and 15% during the long period **(Figure 4a)**. The ITA is a measure of skin pigmentation degree. These results were significantly better than placebo and a visually perceivable improvement of dark circle color was clear to see **(Figure 4b)**.

Seanactiv also reduced the roughness of the skin surface. This was measured in terms of the mean surface texture (Spq) value, which decreased by 14% during the short period and 12% during the long period. These results were significantly better than placebo ($p<0.01$) and a visually perceivable improvement in the appearance of crow's feet wrinkles was clear to see (data not shown).

Taken together, these *in vivo* test results show that applying an eye cream containing 1% Seanactiv contributes significantly to the reduction of the appearance of dark circles and crow's feet wrinkles.

Improving skin luminosity

The same *in vivo* tests also investigated the capacity for Seanactiv to improve the overall appearance of the eye contour by increasing skin luminosity. This involved measuring the lightness of skin color, denoted as the L value. Seanactiv increased the L value by 1.7% during the short period and 2.6% during the long period **(Figure 5a)**. An increase in this value indicates lighter skin color. These results were significantly better than placebo ($p<0.001$) and a visually perceivable improvement in skin luminosity was clear to see **(Figure 5b)**. This indicates that applying eye cream containing 1% Seanactiv contributes to significantly increasing skin luminosity from the first week, compared to the placebo and baseline.

Conclusion

Seanactiv is a new bioactive ingredient from BASF that uses an alga extract rich in fucoidan to quickly help diminish signs of eye-fatigue and aging of the eye area. The solution is 99.8%

Fig. 4b Illustrative VISIA pictures of the dark circle improvement with an eye cream containing 1% of Seanactiv or a placebo formula.

from natural origin and provides a three-step action to revitalize the eye area with visible results within just one week of application. This enables companies that manufacture cosmetic and skin care products to meet rising consumer demand for solutions to address their concerns about signs of aging and tiredness such as dark circles, crow's feet, and dull skin.

In vitro testing has shown that Seanactiv is able to reinforce cytoprotection and limit the appearance of dark circles under the eyes by stimulating synthesis of HO-1 by human dermal keratinocytes by 413% and 598% at 0.02% and 0.06% respectively. Furthermore, *in vitro* tests have proven its efficacy in boosting synthesis of Collagen I by human dermal fibroblasts by 485% and limiting the appearance of wrinkles. It has also demonstrated its capacity to increase synthesis of SIRT-1 by 42%, while stimulating ATP production and mitochondrial activity by 258% and by 138% respectively. *In vivo* tests have demonstrated that Seanactiv is able to reduce the appearance of dark circles and crow's feet wrinkles, while significantly increasing skin luminosity from the first week.

Altogether, these results show that Seanactiv harnesses the power of fucoidan to visibly improve key aspects of eye contour appearance quickly and effectively within just one week.

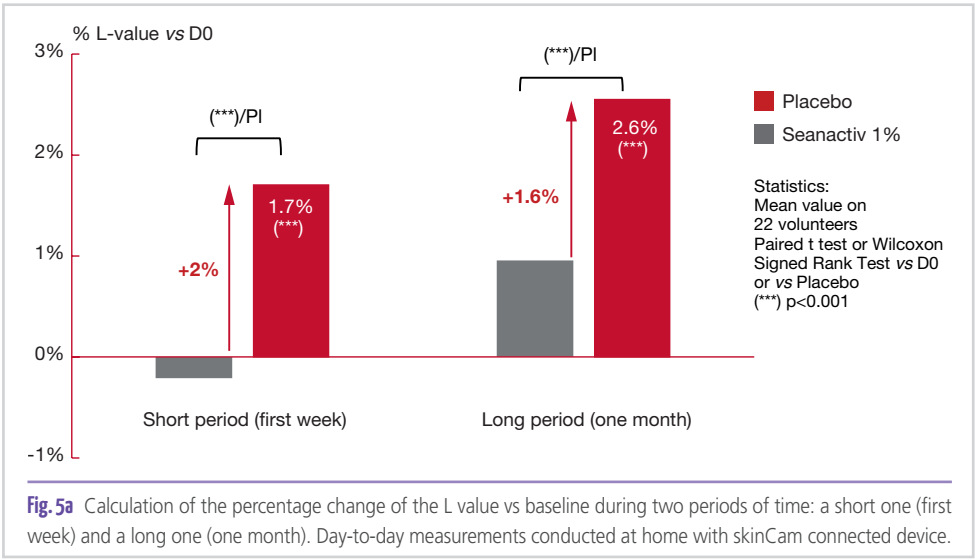

Fig. 5a Calculation of the percentage change of the L value vs baseline during two periods of time: a short one (first week) and a long one (one month). Day-to-day measurements conducted at home with skinCam connected device.

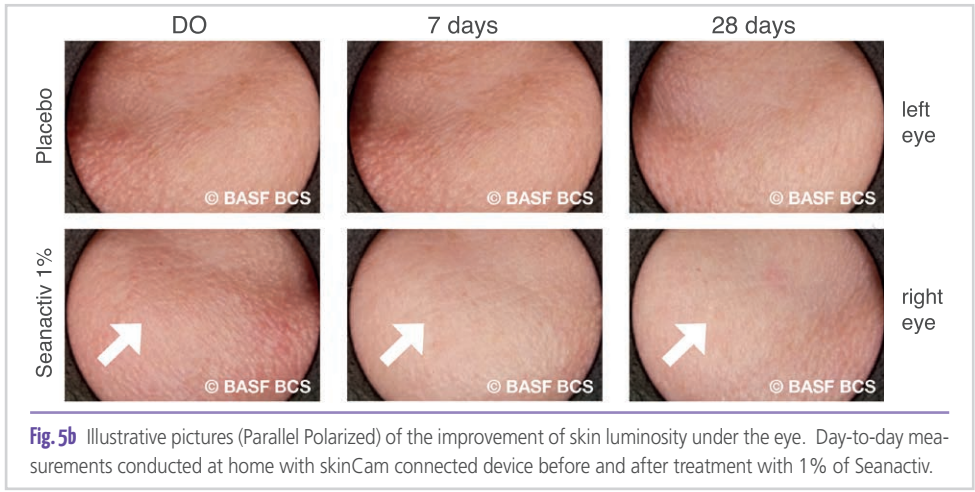

Fig. 5b Illustrative pictures (Parallel Polarized) of the improvement of skin luminosity under the eye. Day-to-day measurements conducted at home with skinCam connected device before and after treatment with 1% of Seanactiv.

References:

[1] *Kwart, D.G., Foulsham, T. and Kingstone, A.* Age and beauty are in the eye of the beholder. Perception 41, 925–938 (2012).

[2] *E. Merinville E, Grennan G. Z , Gillbro J. M, Mathieu J, and Mavon A,* Influence of facial skin ageing characteristics on the perceived age in a Russian female population. International Journal of Cosmetic Science, 37 (Suppl. 1), 3–8; 2015.

[3] *Nkenge, A., Bertin, C., Stamatas, G.N., Giron, A., Rossi, A., Issachar, N. and Fertil, B.* Influence of facial skin attributes on the perceived age of Caucasian women. J. Eur. Acad. Dermatol. Venereol. 22, 982–991; 2008.

[4] *Christensen K, Thinggaard M, McGue M, Rexbye H, Hjelmborg JV, Aviv A, Gunn D, van der Ouderaa F, Vaupel JW.* Perceived age as clinically useful biomarker of ageing: cohort study. BMJ. 339: b5262, 2009.

[5] *Flament F, Pierre J, Delhommeau K, Adam AS.* How a working day-induced-tiredness may alter some facial signs in differently-aged Caucasian women. Int J Cosmet Sci. 2017 39(5):467-475, 2017.

[6] Mintel, 2020: Redefine beauty with the microbiome – Available at: https://clients.mintel.com/insight/redefine-beauty-with-the-microbiome?fromSearch=%3Ffreetext%3Ddark%2520circles

[7] *Fletcher HR, Biller P, Ross AB, Adams JMM.* The seasonal variation of fucoidan within three species of brown macroalgae. Algal research 22, 79-86, 2017.

[8] *Maynard S, Keijzers G, Gram M, Desler C, Bendix L, Budtz-Jørgensen E, Molbo D, Croteau DL, Osler M, Stevnsner T, Rasmussen LJ, Dela F, Avlund K, Bohr VA.* Relationships between human vitality and mitochondrial respiratory parameters, reactive oxygen species production and dNTP levels in peripheral blood mononuclear cells. Aging (Albany NY). 5(11):850-64. doi: 10.18632/aging.100618. 2013.

[9] *Shecterle LM, St Cyr JA.* Dermal benefits of topical D-ribose. Clin Cosmet Investig Dermatol. 2:151-2. doi: 10.2147/ccid.s7487. 2009.

Formulations examples:

CC-Eye perfector (CC-FR-21-BC-50872-05) - *see page 56*

Silky Eye Concentrate (SC-FR-21-BC-50922-01) - *see page 57*

authors

Corinne Reymermier | corinne.reymermier@basf.com
Nicolas Pelletier | nicolas.pelletier@basf.com
Louis Danoux | louis.danoux@basf.com
Wendy Chan | wendy.k.chan@basf.com
Carole Boury | carole.boury@basf.com
Valérie André | valerie.andre-frei@basf.com

www.carecreations.basf.com

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Edelweiss Helps to Protect Against Glycation-related Skin Ageing

F. Paul, D. Imfeld

abstract

Glycation-induced, Advanced Glycation End Products (AGEs) make a significant contribution to signs of skin ageing. In the pro-ageing skin care market, cosmetic antioxidants are often exploited for their ability to protect against free radicals and oxidative stress, but some of these ingredients also have potential to reduce or prevent the effects of glycation. DSM has conducted two new studies to explore the anti-glycation potential in a bioactive with proven antioxidant properties, ALPAFLOR® EDELWEISS CB [INCI *Leontopodium alpinum extract*] an extract of organically grown and ethically sourced Edelweiss plants. The first study demonstrated the ingredient's ability to down-regulate accumulation of AGEs by up to 55% in skin cells *in vitro* and to activate the detoxifying enzyme named Gloxalase-1 (Glo-1), which plays a key role in protecting keratinocyte proteins against oxidative damage during skin ageing, by 65%. The second study demonstrated that the bioactive inhibited Collagen Type IV glycation by 67%.

Antioxidants in pro-ageing skincare - a burgeoning market

With a growing ageing population around the world, there is a high and increasing consumer demand for pro-ageing skin care solutions, particularly those that target the appearance of fine lines and wrinkles. The market for cosmetic antioxidants is gaining noticeable traction [1,2]. In consumers' minds, these ingredients are strongly associated both with naturals and plant extracts (perceived as being cleaner and more trustworthy and environmentally friendly) and reducing signs of skin ageing caused by external and environmental factors. When it comes to the mode of action that delivers these skin care benefits, the focus of antioxidant effects tends to be on protecting against the negative impact of free radicals. However, a process known as glycation, which is less well-known to consumers, also plays a role in the formation of fine lines and wrinkles and antioxidants can help counter this process too.

Glycation, AGEs, and their impact on skin

Glycation is a basic mechanism involved in ageing in humans. It is a non-enzymatic reaction that occurs spontaneously between sugars and proteins, leading to the formation of Advanced Glycation End Products (AGEs), a complex that impairs the function of these proteins and weakens their structure.

The glycation process and the accumulation of AGEs accelerate with lifetime, and in skin, the visible impact begins to manifest from about the age of 35 years. Here, AGEs build up in the extracellular matrix of both the dermis and epidermis. In the dermis, they bind with and alter components such as elastin and collagen, decreasing skin elasticity, increasing rigidity, and leading to signs of ageing such as stiffness and the appearance of lines and wrinkles. Accumulation of glycated proteins in the extracellular matrix is also responsible

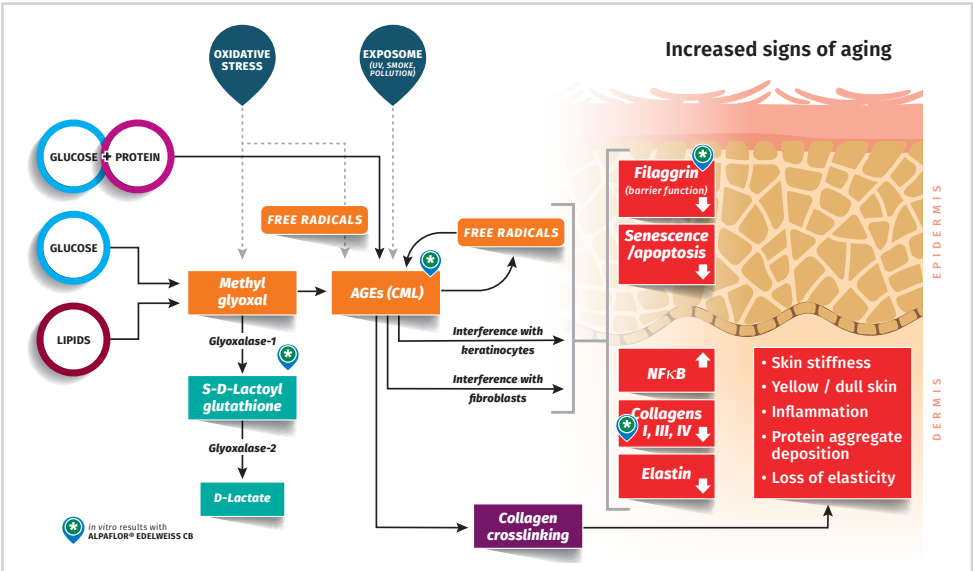

Fig. 1 Glycation processes and their impact on skin

for making the skin dull and yellowish [3]. In the epidermis, *in vitro* studies show that AGEs disrupt the proliferation of keratinocytes [4], making the skin's repair processes less efficient, and impair the skin barrier. Additionally, an accumulation of AGEs in the *stratum corneum* has a negative impact on skin texture which can cause the face to appear older.

Glycation and the formation and accumulation of AGEs are processes that accelerate with time due to an age-associated increase in oxidative stress [5]. Exposure to UV light further aggravates the formation of AGEs, weakening the skin's natural defence systems against reactive oxygen species which in turn results in signs of skin ageing **(Figure 1)**.

Could antioxidants counteract the effects of AGEs?

Given what is known about the impact of AGEs on skin, it is reasonable to assume that cosmetic ingredients that target glycation could help enhance skin appearance. To this end, at DSM, we decided to investigate the anti-glycation potential in an established skin care active already proven for its antioxidant, radical scavenging properties. *Leontopodium alpinum extract* [commercial name ALPAFLOR® EDELWEISS CB].

For our first study, we assessed, *in vitro*, the anti-glycation potential of our active on human epidermal keratinocytes exposed to glycation stress. For the second, we assessed the active's modulating effect on Collagen Type IV Glycation.

Study 1

in vitro assessment of *Leontopodium alpinum* extract's anti-glycation potential in human keratinocytes (NHEK)

Our approach in this study was to measure N^{ε}-(carboxymethyl) lysine (CML), filaggrin (FLG) expression and glyoxalase 1 (Glo1) activity in keratinocytes exposed to glycation stress.

In vitro cell cultures

For all three parts of our study, primary human epidermal keratinocyte NHEK cells from abdominal skin were grown in KGM-Gold™ medium Keratinocyte Growth Medium bullet-kit™ (Lonza).

Anti-glycation potential

The principle of the assay was to evaluate biomarkers of glycation in epidermal keratinocytes activated by glycation stress (by glyceraldehyde). To this end, N^{ε}-(carboxymethyl) lysine (CML) protein adducts, FLG expression, and Glo1 activity in human epidermal keratinocytes NHEK in response to DL-glyceraldehyde (GLA)-induced glycation stress were evaluated

in untreated (control) cultures and cultures treated with the plant extract.

The dry Edelweiss extract was tested at 3 concentrations:

C_1 = 5 µg/ml, C_2 = 25 µg/ml and C_3 = 50 µg/ml

2 control groups were assessed in parallel:

- 1 unglycated control: culture medium only
- 1 glycated control: culture medium + GLA at 500µM

The incubation time under stress conditions (GLA) was 48 hours and the experiments were repeated in triplicate.

1) Intracellular accumulation of CMLs

N^{ε}-carboxymethyllysine was measured in cell extracts of untreated (control) and treated cultures using an Elisa assay kit (OXISELECT™ N^{ε}-(CARBOXYMETHYL)LYSINE (CML) COMPETITIVE ELISA KIT (CELL BIOLABS INC®). Absorbance was recorded at 450nm and calibrated to a CML standard curve. Total protein levels were also determined with a BCA Protein Assay kit (PIERCE™). CML levels were standardized to the protein content of NHEK cultures.

Findings - Control cultures

Glyceraldehyde (GLA) exposure caused a pronounced increase in CML protein adducts. A statistically significant ($p \leq 0.01$, Student's t test) 8-fold induction in basal glycation level was recorded in glyceraldehyde-exposed, untreated control (+)GLA cells.

Findings - *Leontopodium alpinum extract* treated cultures

The intracellular CML accumulation at the end of the GLA stress phase in cells treated with the plant extract at 25 µg/ml (40% decrease, $p \leq 0.01$) and 50 µg/ml (55% decrease, $p \leq 0.01$) was significantly lower than in (+)GLA control cells. At 5 µg/ml it was too diluted and a slight increase in CML level was observed (+19%, $p \leq 0.01$). These results indicate that treating NHEK cells with the active decreases accumulation of GLA-induced CML protein adducts dose-dependently by up to 55% **(Figure 2)**.

2) Filaggrin expression

Filaggrin (FLG) was quantified in cell extracts using a sandwich Elisa, "Human Filaggrin ELISA Kit" (CUSABIO®) with absorbance reading at 450nm. FLG levels, calculated by interpolation from the standard curve were expressed in pg of FLG per extract. FLG levels were standardized to the protein content of NHEK cultures in corresponding extracts. The results were expressed in pg of FLG per mg of proteins and in percentages of (+)GLA control.

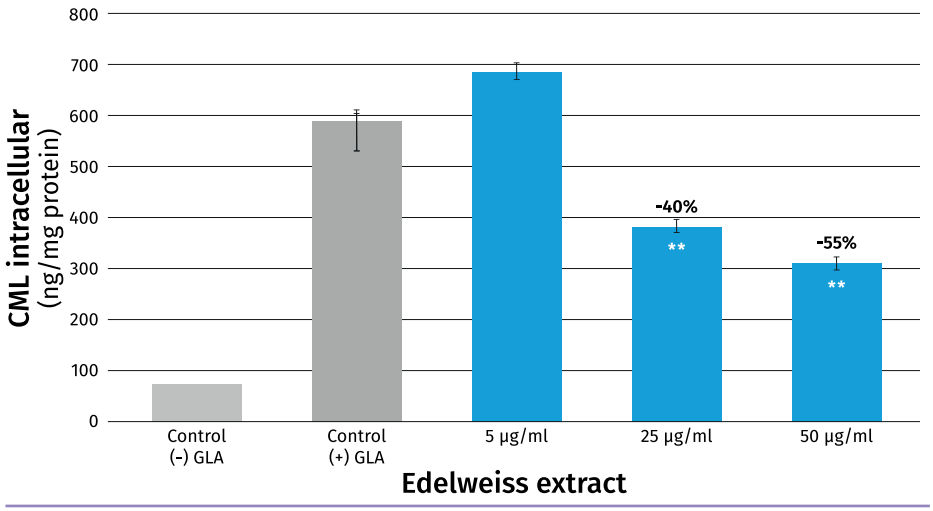

Fig. 2 Intracellular accumulation of CMLs in cultures treated with *Leontopodium alpinum extract* compared to control cultures

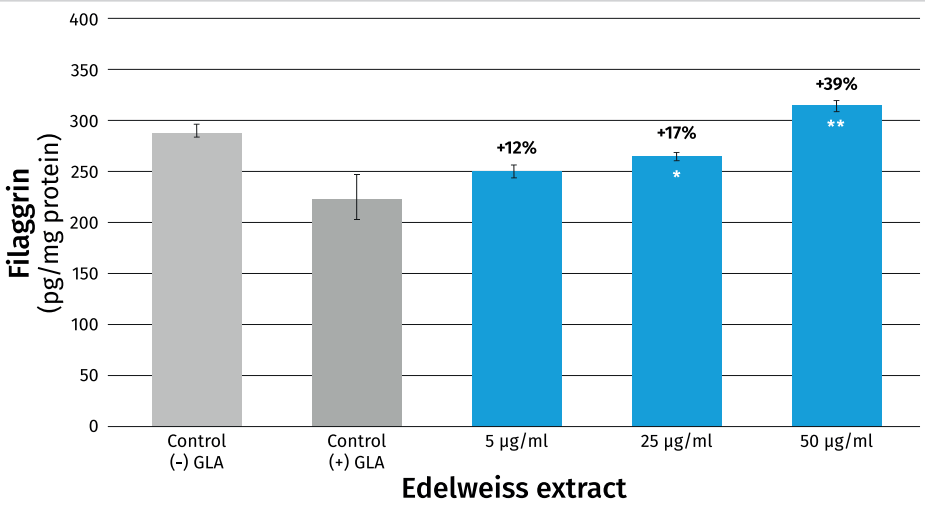

Fig. 3 Expression of Filaggrin in cultures treated with L*eontopodium alpinum extract* compared to control cultures

Findings - Control cultures

Glyceraldehyde (GLA) exposure reduced FLG protein levels in NHEK cells. The down-regulating effect (-23%) was statistically significant ($p \leq 0.01$, Student's t test) compared to unglycated control ((-)GLA) cells.

Findings - *Leontopodium alpinum* extract treated cultures

FLG levels in NHEK cells treated with the plant extract were significantly increased compared to the (+)GLA control **(Figure 3)**. The up-regulating effect appeared dose dependent in the range of tested concentrations and at 25 µg/ml (+17%, $p \leq 0.05$) and 50 µg/ml(+39%, $p \leq 0.01$), the differences proved to be statistically significant (Student t test) compared to the (+)GLA control. Moreover, the +39% increase took FLG levels higher than they were in the non-stressed control cells without GLA.

3) Glyoxalase 1 activity

Cell extracts were assayed for Glyoxalase 1 (Glo1) activity by measuring the rate of formation of S-D-lactoylglutathione from Glo1 substrate hemithioacetal pre-formed by incubation of methylglyoxal (MGO) and glutathione (GSH) spectrophotometrically at 240nm. Glo1 activity is given in units per mg of protein where one unit is the amount of enzyme that catalyses the formation of 1.0 µmole of S-D-lactoylglutathione per minute under assay conditions. The specific activity of Glo1 is calculated and expressed as milliunits (mU) per extract.

Additionally, cell extracts were used to quantify total protein content using a BCA Protein Assay kit.

Specific activities were standardized to the protein content of NHEK cultures in corresponding extracts. The results were expressed in mU per mg of proteins and in percentages of (+) GLA control.

Findings - Control cultures

The incubation of NHEK cultures with 500µM glyceraldehyde for 48h markedly increased Glo 1 activity. A statistically significant ($p \leq 0.01$, Student's t test) 2.8-fold induction in basal Glo1 activity was recorded in glyceraldehyde-exposed untreated control (+) GLA cells.

Findings - *Leontopodium alpinum extract* treated cultures

The Glo1 activity of cells treated with the Edelweiss extract in the presence of GLA were further and markedly increased compared to the (+)GLA control **(Figure 4)**. The up-regulating effect was dose dependent in the range of tested concentrations. At 25 µg/ml it was +32%, ($p \leq 0.05$) and at 50 µg/ml it was +65%, ($p \leq 0.01$). Both increases are statistically significant (Student's t-test).

Study 2

Assessment of the modulating effect of *Leontopodium alpinum extract* on Collagen Type IV Glycation

For this study, we followed an in tubo experimental approach based on the time course formation of N^{ε}-(carboxy -methyl) lysine (CML) in collagen type IV. The assay system involved a tissue culture plate coated with collagen IV (Corning® Bio-Coat™ Collagen IV Cultureware).

Antiglycation activity

To evaluate our Edelweiss extract's anti-glycation properties, we measured the formation rate of AGEs after incubation of collagen type IV with D-ribose. The AGE level was evaluated by measuring the amount of N^{ε}-carboxymethyllysine (CML) in collagen.

A 0.25M D-ribose solution in phosphate-buffered saline (PBS) (pH7.4) was added to each well of a 24-well plate coated with collagen IV (Corning®) under sterile conditions. The plates were then maintained at 37°C and 5% CO_2 for 15 days.

At the end of the incubation period, the plates were washed several times with PBS to remove excess ribose. After wash-

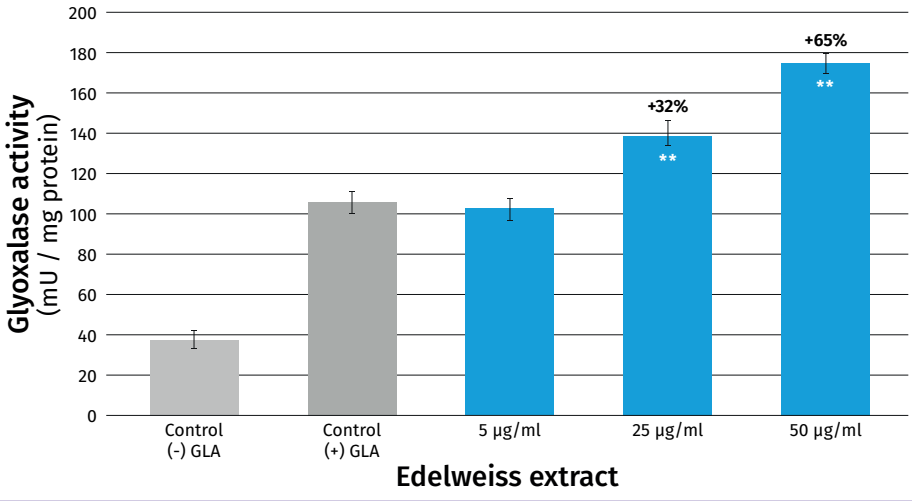

Fig. 4 Glyoxalase 1 activity in cultures treated with Leontopodium alpinum extract compared to control cultures

ing, N^{ε}-carboxymethyllysine was measured by means of a non-competitive ELISA assay carried out directly in the wells of the plate coated with collagen IV.

The wells were blocked with 1% BSA in washing buffer (0.05% Tween 20 in phosphate-buffered saline (PBS-T)) at room temperature (RT) for 1 hour and then incubated with anti-CML polyclonal antibody (Antibodies-online GmbH) at RT for 2 hours After 5 washes with PBS-T buffer, the wells were incubated with alkaline phosphatase-conjugated goat anti-rabbit IgG at RT for 1hour. After incubation, the wells were washed and incubated in PNPP (para-nitrophenylphosphate) substrate solution. The absorbance of the final p-nitrophenol reaction product was measured at 405 nm.

The Edelweiss extract was tested at five concentrations from 3.125 to 50µg/ml (see **Figure 5**). As a control, a blank [(-)RIB] was performed by preparing PBS without ribose.

Each experimental condition was run in triplicate (n=3).

Expression of results

The absorbance of test-solutions (OD) was corrected by subtracting the absorbance of the blank [(-)RIB] (control collagen IV well incubated without ribose and without Edelweiss extract). The AGE_{CML} level was calculated as follows:

$$[AGE_{CML}\ \%] = [OD_{cor}.\ Treated / OD_{cor}.\ Control] \times 100$$
$$\text{with } OD_{cor} = OD_{[(+)RIB]} - OD_{[(-)RIB]}.$$

The results were expressed as a corrected OD unit (OD_{cor}] or in percentages of the control or solvent control.

Findings – control wells

Our results showed that ribose (RIB) exposure markedly increased CML formation. A statistically significant ($p \leq 0.01$, Student's t test) 2.4-fold induction in basal glycation level was recorded in ribose-exposed (+) GLA collagen.

Findings – treated wells

The results [AGE_{CML} %] of the assay were collected in table format. The data was analysed statistically using the Student's t test.

Our results showed that in the presence of *Leontopodium alpinum* extract, there was a pronounced decrease of up to 67% in glycated Collagen IV, indicating the active's anti-glycation potential. The inhibition was dose-dependent in the range of the tested concentrations **(Figure 5)**.

The IC_{50} value was calculated by linear regression

[Inhib. (%)/(log(Conc.)]: IC_{50} = 28 µg/ml.

Conclusions

Study 1

Under the experimental conditions described above, to begin, our first study showed that exposure of NHEK cells to glyceraldehyde led to an intracellular accumulation of CMLs, a reduction in FLG and an increase in Glo 1 activity.

Next, our results indicated that in the range of the tested concentrations (5–50µg/ml), our plant extract reduced glyceraldehyde-induced formation of CML in a dose-dependent manner. Concurrently, and under the same experimental conditions, the plant extract significantly increased the Glo1 activity of cells in a dose dependent manner.

Finally, in the range of the tested concentrations (5–50µg/ml), the plant extract demonstrated the capacity to alleviate a glyceraldehyde-induced decrease in FLG in a dose dependent manner. It is also worth noting that the highest tested concentration (50µg/ml) showed the ability to restore barrier function impaired by glyceraldehyde completely.

Study 2

Under the experimental conditions described above, the results of our second study evidenced that the Edelweiss extract

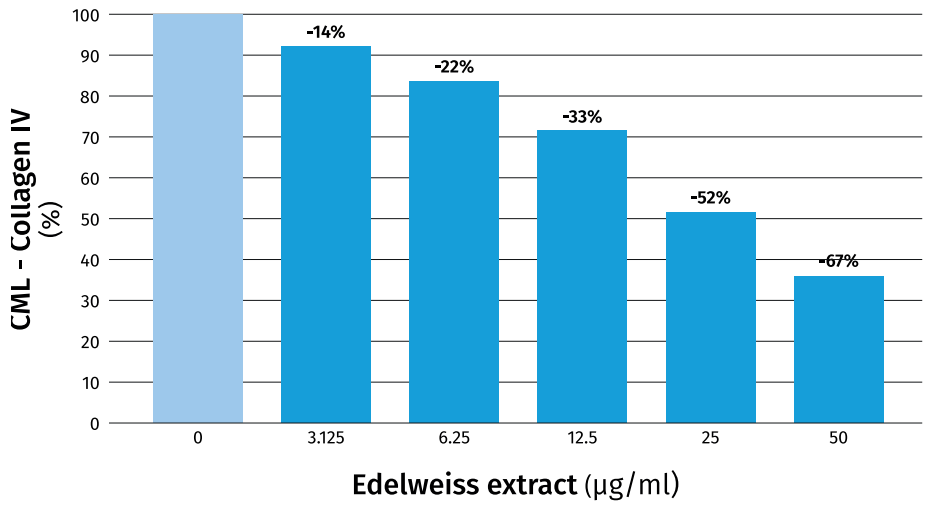

Fig. 5 Reduction of CML induction in wells treated with Leontopodium alpinum

inhibited glycation reaction, and sustained dose dependency. The IC_{50} value was equal to 28 µg/ml.

Overall conclusion

The Edelweiss extract shows anti-glycation properties through:

- its ability to significantly down-regulate the accumulation of AGEs in skin cells which show toxic effects resulting in cell dysfunction during ageing,
- Glo 1 activation which plays a key role in the detoxification of dicarbonyls and in the protection of keratinocyte proteins against oxidative damage during skin ageing,
- its inhibiting effect on Collagen Type IV glycation.

Furthermore, it is worth noting that anti-glycation potential is associated with a protective effect on "skin barrier function" which is a key element of cellular homeostasis during skin ageing.

Therefore, in addition to its proven ability to protect skin from oxidative stress, *Leontopodium alpinum* extract also demonstrates potential to protect against glycation, further enhancing its ability to help deliver skin care benefits such as healthy-looking skin, slowing down the appearance of visible signs of skin ageing, and preserving collagen integrity for smooth, elastic and rejuvenated skin.

About the active

Leontopodium alpinum extract [commercial name ALPAFLOR® EDELWEISS CB] is produced from a unique Edelweiss variety, *Leontopodium alpinum 'Helvetia'*. It is extracted from particularly robust plants cultivated at altitudes of up to 3000 metres and that develop an exceptionally high active content of leontopodic acid and flavonoids due to the high levels of UV radiation, strong winds and temperature extremes they are exposed to.

Its antioxidant properties and protective action on the skin barrier have made it a popular choice in pro-ageing skin care formulations. Additionally, it meets growing consumer expectations for natural and ethically sourced products as it is extracted from organically grown plants sourced through a short, local, and fair supply chain. Furthermore, because the Edelweiss is a particularly resilient plant, *Leontopodium alpinum extract* is likely to have a symbolic appeal to consumers turning to sustainable ingredients as part of a drive to boost personal resilience and contribute to a more responsible consumption as part of the rapidly growing, post-Covid 19 trend for "resilient beauty".

Acknowledgements:

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References:

[1] Frost and Sullivan report on the future of the Western European market the cosmeceuticals 2018

[2] GNPD MINTEL with PCA CMI analysis

[3] *Yuki Ogura, Tomohiro Kuwahara, Minoru Akiyama, Shingo Tajima, Kazuhisa Hattori, Kouhei Okamoto, Shinpei Okawa, Yukio Yamada, Hachiro Tagami, Motoji Takahashi, Tetsuji Hira* (2011) Dermal carbonyl modification is related to the yellowish color change of photo-aged Japanese facial skin, J. of Dermatological Science 64(1): 45-52

[4] *Zhu P, Yang C, Chen LH, Ren M, Lao GJ, Yan L* (2011). Impairment of human keratinocyte mobility and proliferation by advanced glycation end products modified BSA. Arch Dermatol Res, 303:339-50

[5] *Uribarri J, Cai W, Peppa M, Goodman S, Ferrucci L, Striker G, et al* (2007). Circulating glycotoxins and dietary advanced glycation end products: two links to inflammatory response, oxidative stress, and aging. J Gerontol A Biol Sci Med Sci, 62:427-33.

authors

François Paul
Global Marketing Manager Skin Actives and Vitamins & Site Manager Vouvry

Dominik Imfeld
Head of Claim Substantiation Skin Actives

DSM Nutritional Products Ltd., Switzerland
www.dsm.com

A Natural Anti-acne Care for Adults

M. Coirier, M. Mangier, E. Lasjaunias, E. Aymard, B. Closs

abstract

Acne is a chronic multifactor pathology characterized by the appearance of non-inflammatory and inflammatory lesions. It is one of the most frequent reasons for appointments with dermatologists (20% of consultations). Acne of teenagers normally disappears around the age of 20 but the symptoms can continue into adulthood.

To tackle this pathology, SILAB developed ACNESIUM®, a purified natural active ingredient obtained from the pericarps of pomegranate (*Punica granatum*), able to prevent the clinical signs of slight to moderate acne in adults.

Its clinical efficacy in a cohort of Caucasian adult patients with acneic skin has been demonstrated under control by dermatologists, revealing that ACNESIUM® is therapeutically effective in the daily care of slight to moderate acne in Caucasian adults.

Introduction

Acne is a chronic multifactor pathology characterized by the appearance of non-inflammatory lesions called retentional lesions (open and closed comedones) and inflammatory lesions (papules, pustules, nodules and cysts). Although acne affects mostly teenagers, recent epidemiological studies have confirmed its substantial prevalence in adult women [1]. The latter can take on three different forms: acne persisting since adolescence, acne occurring for the first time in adults, and a relapse of acne having disappeared in teenagers and reappearing in adults [2]. Several factors are involved in its appearance or aggravation: genetics, hormone changes or lifestyle [3]. Beyond its incidence on the skin, acne has a major impact of the quality of life of those afflicted with the disorder. The pathology is localized primarily on the face and therefore strongly affects a person's appearance, intimately linked to self-esteem and self-confidence. Depression, suicidal thoughts, anxiety and social inhibition are the daily burden of acneic subjects [2].

From a biological standpoint, the appearance of acne lesions results from the dysfunction of several mechanisms in the pilosebaceous follicle. Four biological components are thus modified. First one is the modification of sebaceous gland activity characterized by quantitative and qualitative modifications of sebum. Sebaceous gland metabolism is activated by a number of factors, both endogenous, e.g. hormones, *Cutibacterium acnes*, level of lipophagic activity, and exogenous, *e.g.* stress. This causes a modification of the lipid profile of sebum and its abnormally high production.

The second biological event occurring early in the development of acne lesions is hyperkeratinization. It is characterized by abnormal proliferation and keratinization of follicle keratinocytes that cause obstruction of the excreting canal. Excess sebum produced cannot be evacuated and so the pilosebaceous canal becomes dilated. The first clinical signs of acne appear: microcomedones, lesions invisible to the naked eye.

Also, modification of the microbiota especially the bacterial species *C. acnes* is considered to be one of the major pathogenic factors contributing to the development of acne. As a result of the high quantity of sebum and the low oxygen concentration, the microcomedones is an environment favorable to the growth of this microorganism. Recent studies have led to the identification of several sub-types of *C. acnes*, called phylotypes, with variable degrees of virulence. In cases of acne, a loss of phylotype diversity of *C. acnes* with a shift to phylotype IA1 has been observed. Phylotype IA1 bacteria can communicate using a system called quorum sensing, thereby favoring the expression of virulence factors, *e.g.* formation of biofilms, required for their survival.

The fourth biological component is inflammation, a biological process present throughout the formation of acne lesions. *C. acnes* bacterium stimulates the production of pro-inflammatory mediators by several cell types of the pilosebaceous unit (keratinocytes, sebocytes, immune cells), thereby leading to the appearance of inflammatory lesions (papules, pustules, nodules and cysts). This inflammatory phenomenon can be exacerbated by a variety of exogenous and endogenous factors, *e.g.* hormones, stress and a modification of the composition of sebum.

Faced with these elements, SILAB developed ACNESIUM®, a purified natural active ingredient able to restore the homeosta-

sis of acneic skin. Indeed, an innovative 3D *in vitro* model [4] demonstrated the efficacy of ACNESIUM® in targeting the previous-mentioned four main anomalies (*data not shown in this publication*). At the level of individuals, it leads to a considerable improvement of clinical signs and patient's quality of life, as shown by the clinical studies in Caucasian adults with slight to moderate acne, demonstrated in this publication.

1. Material and methods

1.1. Objective

The clinical study was conducted in Spain between April 2019 and February 2020 to determine the effect of ACNESIUM® formulated at 0.5% in an emulsified gel as a care product for the treatment of the skin of Caucasian adults with slight to moderate acne. The effect was evaluated after 30 days of twice daily application in comparison to a placebo formula.

1.2. Panel of patients and inclusion criteria

This study was outsourced to ClinReal Online (Toulouse, France). It was conducted with 12 dermatologists in Spain, involving 92 Caucasian adults seen in doctor's offices in the context of consultations.

To be included in the study, subjects had to be phototype I to V female subject between 25 and 40 years of age with slight to moderate acne on the face (GEA score 2 or 3), with more than 5 retentional lesions and more than 5 inflammatory lesions, but without nodules or cysts on the face. Not requiring the prescription of an oral anti-acne drug product was another criterion for inclusion. Patients treated with a topical anti-acne drug product on the face for the past 2 weeks or an oral anti-acne drug product for the past 4 weeks were excluded as well as patients having been treated with isotretinoin for the past 3 months. Subjects also had to agree to not modify any hormone or contraceptive products being taken throughout the entire duration of the study. Changing personal hygiene routines throughout the entire duration of the study was forbidden. The last criterion was to have no history of allergy or hypersensitivity reaction to a cosmetic product or to the ingredients in the products provided for the study.

The subjects were placed in 2 groups. The placebo group included 46 women between 25 and 41 years of age (mean age 30 years) and the ACNESIUM® group included 46 women between 25 and 48 years of age (mean age 31 years)*.

1.3. Treatment conditions

Subjects applied either the placebo formula or the formula containing ACNESIUM® for a maximum of 30 days.

* With a derogation on the age for five subjects distributed in the two groups

The creams tested were formulated, packaged and identified by SILAB. The INCI of the placebo formula was: Aqua (Water), Cetearyl Ethylhexanoate, Propanediol, Arachidyl Alcohol, Behenyl Alcohol, Polyacrylamide, Arachidyl Glucoside, 1,2-Hexanediol, Caprylyl Glycol, Cetyl Alcohol, C13-14 Isoparaffin, Maltodextrin, Laureth-7. The INCI of the formula containing ACNESIUM® was: Aqua (Water), Cetearyl Ethylhexanoate, Propanediol, Arachidyl Alcohol, Behenyl Alcohol, Polyacrylamide, Arachidyl Glucoside, 1,2-Hexanediol, Caprylyl Glycol, Cetyl Alcohol, C13-14 Isoparaffin, Maltodextrin, Laureth-7, Punica Granatum Pericarp Extract.

Each product was applied to the entire face in the morning and evening for 1 month. Before application, facial skin was cleansed with the usual hygiene measures that were to be used for the entire duration of the study. No other cosmetic or drug product for the same indications was to be used during the treatment and the subjects were required to maintain their usual hygiene measures and lifestyle.

1.4. Criteria of efficacy

The clinical efficacy of the treatment was assessed after 30 days of application, based on an evaluation by the dermatologists (counting lesions, scoring acne severity and overall appreciation of the care product) and an evaluation by the patients (evaluation of quality of life, self-evaluation of efficacy and overall appreciation of the care product).

Evaluation by dermatologists

The primary efficacy variable observed by the dermatologists was to count the precise number of acne lesions on the face (open comedones, closed comedones, papules, pustules, nodules and cysts) and to score the severity of the acne using the GEA scale (Global evaluation acne). This scale was created and validated in 2011 by a French team [5] and is the reference tool for the overall evaluation of the severity of the pathology by dermatologists.

The GEA scale is composed of six stages shown in **Table 1**. A reduction of the GEA score shows the clinical efficacy of the treatment tested.

This was complemented by an overall appreciation of the product determined by the dermatologists after 30 days of twice daily application, using a questionnaire. It included the seven following statements: 1. The product favors the reduction of inflammatory lesions; 2. The product favors the reduction of retentional lesions; 3. The product attenuates shininess of the skin; 4. The product attenuates dilated pores; 5. The product has a hydrating effect; 6. The product can be used to treat slight to moderate acne; 7. The product is satisfying overall. There were five possible answers to the previous-mentioned items: agree; mostly agree; mostly disagree; disagree; no opinion.

Evaluation by subjects

Secondary efficacy endpoints included the evaluation of the quality of life by subjects (the Acne-QoL quality of life questionnaire [6]) to be filled on D0, and D30. This questionnaire enables the impact of facial acne on the subject's quality of life to be evaluated. It is suited to evaluate quality of life in the context of a treatment because the questions concern the previous week. It contains 19 items in a context of facial acne, organized as four indices: self-perception, emotional, social and symptoms of acne. The self-perception index involves questions 1, 2, 3, 6 and 10 (1. How unattractive did you feel?; 2. How embarrassed did you feel?; 3. How self-conscious (uneasy about yourself) did you feel?; 6. How dissatisfied with your self-appearance did you feel?; 10. How much was your self-confidence (sure of yourself) negatively affected?). The emotional index involves questions 4, 5, 7, 8 and 9 (4. How upset were you about having facial acne?; 5. How annoyed did you feel at having to spend time every day cleaning and treating your face?; 7. How concerned or worried were you about not looking your best?; 8. How concerned or worried were you that your acne medication/products were working fast enough in clearing up the acne on your face?; 9. How bothered did you feel about the need to always have medication or cover-up available for the acne on your face?). The social index involves questions 11, 12, 13 and 14 (11. How concerned or worried were you about meeting new people?; 12. How concerned or worried were you about going out in public?; 13. How much was socializing with people a problem for you?; 14. How much was interacting with the opposite sex a problem for you?). The symptoms of acne index involves questions 15, 16, 17, 18 and 19 (15. How many bumps did you have on your face?; 16. How many bumps full of pus did you have on your face?; 17. How much scabbing from your facial acne did you have?;

0	Clear. No lesions	Residual pigmentation and erythema may be seen
1	Almost clear. Almost no lesions	A few scattered open or closed comedones and very few papules
2	Mild	*Easily recognizable:* less than half of the face is involved. A few open or closed comedones and a few papules and pustules
3	Moderate	*More than half of the face* is involved. Many papules and pustules, many open and closed comedones. One nodule may be present
4	Severe	*Entire face* is involved, covered with many papules and pustules, open or closed comedones and rare nodules
5	Very severe	*Highly inflammatory acne* covering the face with presence of nodules

Table 1: Stages included in the GEA (Global evaluation acne) scale.

18. How concerned or worried were you about scarring from your facial acne?; 19. How oily was your facial skin?).

Each answer was then encoded as follows: extremely = 0, very much = 1, quite a bit = 2, a good bit = 3, somewhat = 4, a little bit = 5 and not at all = 6. An overall quality of life index can be calculated by adding up the above four indices. An increase in the numerical value of these indices during treatment indicates improved quality of life of the subject.

Finally, an overall evaluation of the efficacy of the product was assessed *via* a self-evaluation by the subjects. It was conducted after 30 days of twice daily application using a questionnaire including 22 items divided into two categories, efficacy and overall evaluation. In the efficacy section, the following 14 statements were included: 1. The product reduces inflammatory skin lesions; 2. The product reduces non-inflammatory lesions; 3. The product attenuates the oily appearance of my skin; 4. The product attenuates shininess of my skin; 5. The product reduces marks from pimples; 6. The product makes my pores less visible; 7. The product improves skin relief (smoother and more uniform appearance of my skin); 8. The product results in a more uniform complexion; 9. The product reduces imperfections during my periods; 10. The product does not dry my skin; 11. The product refines the grain of my skin; 12. The product results in a fresher complexion; 13. The product makes my skin clearer; 14. With this product, my skin has fewer lesions. The overall evaluation section contained the following items: 15. The product can be claimed to have a purifying action; 16. The product can be claimed to have an anti-oily skin action; 17. The product can be claimed to have a matifying action; 18. The product can be claimed to have a moisturizing action; 19. My skin has a healthier appearance; 20. I will gladly continue to use this product; 21. The product is satisfying overall; 22. The product has good overall efficacy.

There were five possible answers to items 1 to 21: agree; mostly agree; mostly disagree; disagree; no opinion. Concerning the overall efficacy of the product tested (item 22), subjects were asked to evaluate efficacy with the following scale: very good efficacy, good efficacy, little efficacy, no efficacy.

2. Results

2.1. Evaluation by dermatologists

Total number of lesions

In the conditions of this study, after 30 days of twice daily application on the face, results in the group having used the formula containing ACNESIUM® at 0.5% showed a significant reduction in the total number of lesions by 43.9% *vs.* 34.0% in the group having used the placebo formula **(Figures 1 and 2)**.

Severity of acne (GEA score)

In the same conditions, the severity score of acne decreased by 26.4% in subjects having used the formula containing ACNESIUM® at 0.5% (effect observed in 61% of subjects) and by 17.0% in subjects having used the placebo formula (effect observed in 33% of subjects).

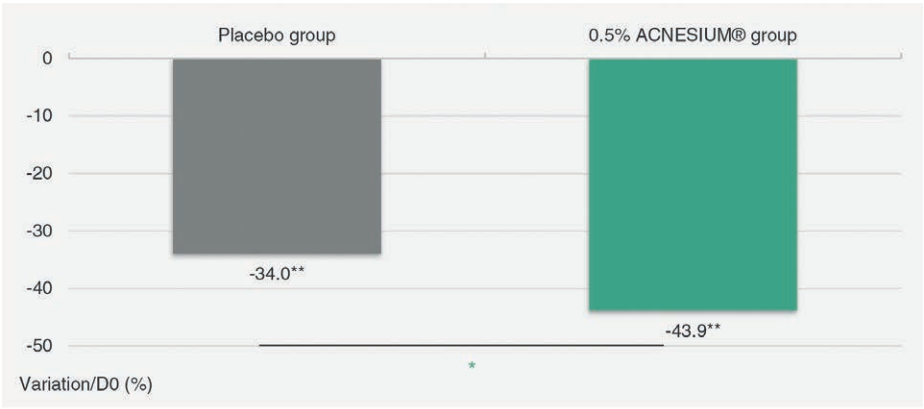


Fig. 1 Change in the total number of lesions *vs.* D0.

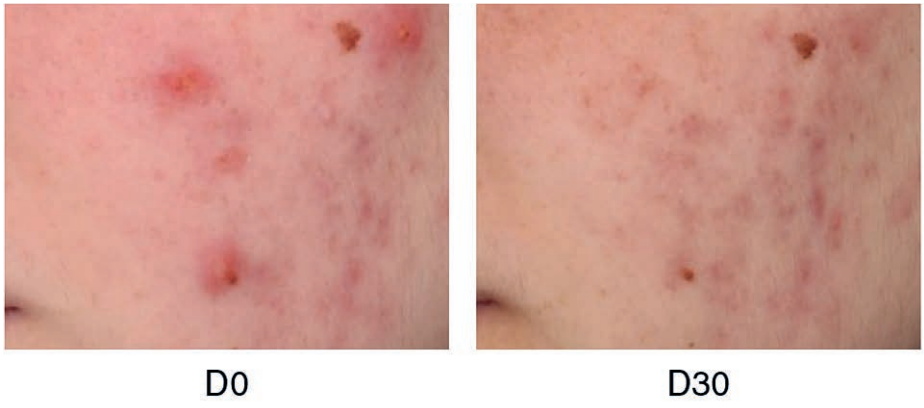
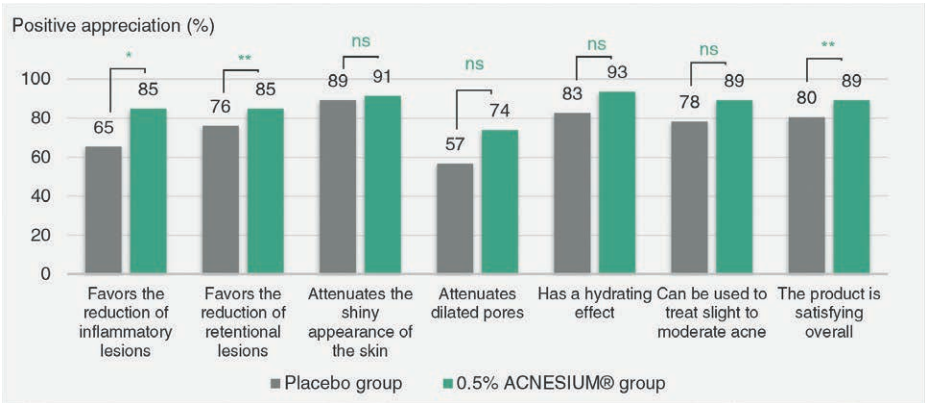


Fig. 2 Effect of ACNESIUM® in an adult Caucasian volunteer after 30 days of treatment.

Overall appreciation

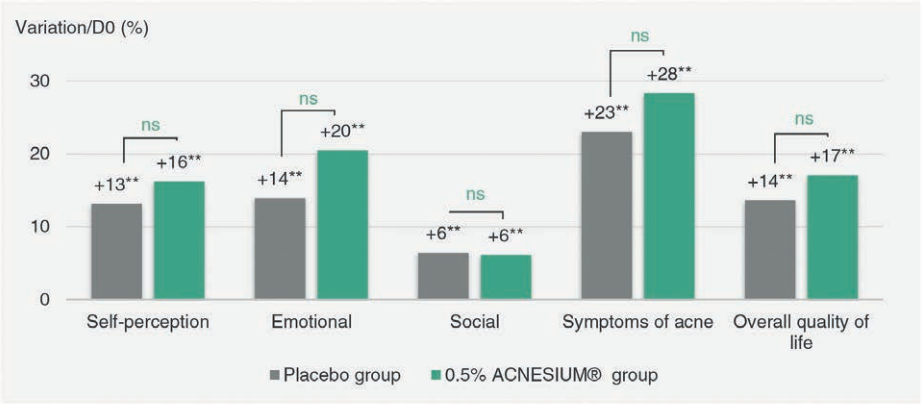
At the end of the study, the evaluations by dermatologists were more positive overall for the group having used ACNESIUM® at 0.5% than for the placebo group.

In particular, the dermatologists believed that the formula containing ACNESIUM® favored the reduction of inflammatory lesions and retentional lesions, and was satisfying overall, with a statistically significant difference compared to the placebo group. Appreciation by dermatologists was also more positive for subjects having used ACNESIUM® regarding the attenuation of the shiny appearance of skin (91% *vs.* 89%), the attenuation of dilated pores (74% *vs.* 57%), the hydrating effect (93% *vs.* 83%) and the possibility of using the product to treat slight to moderate acne (89% *vs.* 78%) **(Figure 3)**.



*: significant difference/placebo (P < 0.05)
**: significant differences/placebo (P < 0.01)
ns: non-significant differences/placebo

Fig. 3 Overall assessment by dermatologists after 30 days of use.



**: significant differences/D0 (P < 0.01)
ns: non-significant differences/placebo

Fig. 4 Change in the indices of quality of life after 30 days of twice daily application.

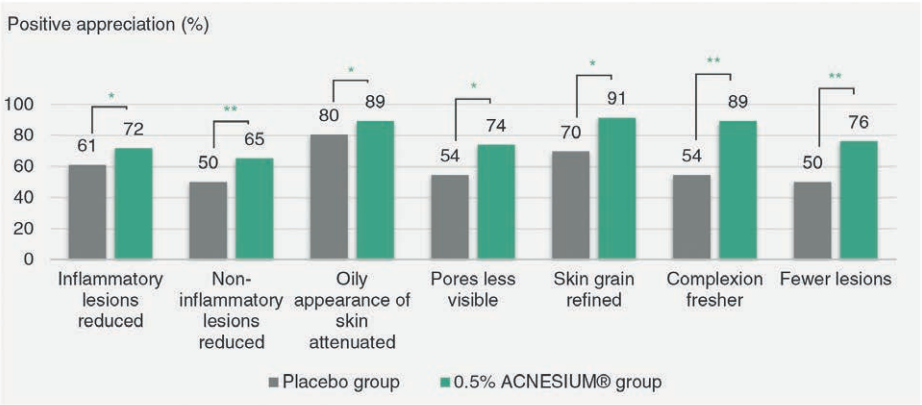
2.2. Evaluation by subjects

Evaluation of quality of life

All the indices of quality of life on D30 compared to D0 were more positive overall in the group having used ACNESIUM® at 0.5% than in the placebo group. A statistically significant difference compared to D0 was noted for the self-perception index (with a 16% increase in the score compared to 13% for the placebo), for the emotional index (with a 20% increase in the score compared to 14% for the placebo), for the symptoms of acne index (with a 28% increase in the score compared to 23% for the placebo) and for the overall quality of life index (with a 17% increase in the score compared to 14% for the placebo) **(Figure 4)**.

Self-evaluation of efficacy

In addition, self-evaluation results confirmed these data. A statistically significant increase in the group having used ACNESIUM® at 0.5% was noted, in particular for the fol-



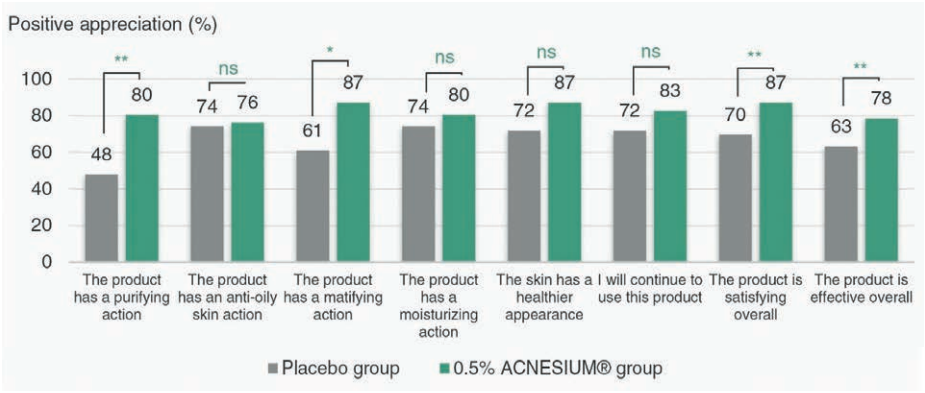
*: significant differences/placebo (P < 0.05)
**: significant differences/placebo (P < 0.01)

Fig. 5 Self-evaluation of efficacy by subjects after 30 days of twice daily application.

lowing items: refines the grain of my skin (91% *vs.* 70%); provides a fresher complexion (89% *vs.* 54%); with this product, my skin has fewer lesions (76% *vs.* 50%) **(Figure 5)**.

Overall appreciation

The overall appreciation by subjects having used ACNESIUM® at 0.5% was also more positive than that by the placebo group. In particular, a statistically significant increase in the group having used ACNESIUM® was noted for the following items: the product can be claimed to have a purifying action (80% *vs.* 48%); the product can be claimed to have a matifying action (87% *vs.* 61%); the product is satisfying overall (87% *vs.* 70%) and the product has good overall efficacy (78% *vs.*63%) **(Figure 6)**.



*: significant difference/placebo (P < 0.05)
**: significant differences/placebo (P < 0.01)
ns: non-significant differences/placebo

Fig. 6 Overall appreciation by subjects after 30 days of twice daily application.

Conclusion

ACNESIUM® is an active ingredient obtained from the pericarps of pomegranates (*Punica granatum*). A targeted study by SILAB has shown indeed that the immature pericarp of pomegranates contains a rich and complex mixture of polyphenols whose interest for the optimal and transversal treatment of slight to moderate acne has been shown.

This study demonstrated the efficacy of this natural active in adult Caucasian women under control by dermatologists. The GEA score (Global evaluation acne) decreases and the number of lesions is significantly reduced. This care combining rapidity of action and efficacy attenuates the pathology symptoms and improves the general aspect of the skin, resulting in enhanced self-perception and self-confidence and in an improved patient's quality of life.

Available in preservative-free powder, it offers optimal skin tolerance. It is totally safe and can be used by adults without risk in leave-on face and body cosmetic and dermo-cosmetic products.

References:

[1] *Dréno, B.* (2015) Treatment of adult female acne: a new challenge. Journal of the European Academy of Dermatology and Venereology: JEADV, 29 Suppl 5, p. 14–19. DOI: 10.1111/jdv.13188.

[2] *Dréno, B.; Bagatin, E.; Blume-Peytavi, U.; Rocha, M.; Gollnick, H.* (2018) Female type of adult acne: Physiological and psychological considerations and management. Journal der Deutschen Dermatologischen Gesellschaft = Journal of the German Society of Dermatology: JDDG, vol. 16, n° 10, p. 1185–1194. DOI: 10.1111/ddg.13664.

[3] *Di Landro, A.; Cazzaniga, S.; Parazzini, F.; Ingordo, V.; Cusano, F.; Atzori, L. et al.*, (2012) Family history, body mass index, selected dietary factors, menstrual history, and risk of moderate to severe acne in adolescents and young adults. Journal of the American Academy of Dermatology, vol. 67, n° 6, p. 1129–1135. DOI: 10.1016/j.jaad.2012.02.018.

[4] *Laclaverie M.; Rouaud-Tinguely P.; Grimaldi C.; Jugé R.; Marchand L.*; Aymard E.; Closs B. (2021) Development and characterization of a 3D in vitro model mimicking acneic skin, Experimental Dermatology, vol. 30, p. 347-357.

[5] *Dréno, B.; Poli, F.; Pawin, H.; Beylot, C.; Faure, M.; Chivot, M.; Auffret, N.; Moyse, D.; Ballanger, F.; Revuz, J.* (2011) Development and evaluation of a Global Acne Severity Scale (GEA Scale) suitable for France and Europe. In : Journal of the European Academy of Dermatology and Venereology : JEADV, vol. 25, n° 1, p. 43–48. DOI: 10.1111/j.1468-3083.2010.03685.x.

[6] *Tan, J.; O'Toole, A.; Zhang, X.; Dréno, B.; Poulin, Y.* (2012) Cultural and linguistic validation of acne-QoL in French. In : Journal of the European Academy of Dermatology and Venereology : JEADV, vol. 26, n° 10, p. 1310–1314. DOI: 10.1111/j.1468-3083.2011.04193.x.

authors

Mélanie Coirier, Mélanie Mangier,
Emilie Lasjaunias, Elodie Aymard, Brigitte Closs

SILAB
19240 Saint-Viance | France

www.silab.fr

Antibacterial, Anti-keratinized and Anti-inflammatory Effects of the GMP, a Multi-herb Extraction on Acne Vulgaris

Yi-na Lu, Guang-yin Wang, Jun Tian

abstract

Recently reports showed that the pathobiology of acne vulgaris was arising from the exploration of sebaceous gland biology, hormonal factors, hyper-keratinization, and the role of bacteria, sebum, nutrition, cytokines and toll-like receptors (TLRs). *Propionibacterium acnes* (*P. acnes*) has a strong proinflammatory activity and targets molecules involved in the innate cutaneous immunity on keratinocytes by acting on TLR-2, leads to the development of comedones. GMP, a multi-herb extraction, targeted most of the major pathogenic features of acne with desired physicochemical traits. It strongly suppressed *P. acnes* growth, and reduced inflammation by suppressing the TLR-2/NF-κB pathway in SZ-95 sebocytes and HaCaT keratinocytes. GMP exhibited a marginal effect on cell viability and may have modulated hyper-keratinization of the epidermis. These results demonstrate the clinical feasibility of applying GMP for the treatment of acne.

Introduction

Acne vulgaris is a chronic inflammatory skin disease which occurs in the follicle and the sebaceous gland unit. It is mainly found in adolescents, and 80 percent of the 11~30 year old people suffer from this disease. Increased sebum production under the inducing factors such as diet, living habits and androgens, abnormal keratinization of keratinocytes, colonization by *Propionibacterium acnes* (*P. acnes*) and inflammation are thought to be the major pathogenic factors. Topical antimicrobials, topical retinoids, oral antibiotics, and oral isotretinoin were used as a commonly therapy for acne, with a limited effect due to skin irradiation and antibiotic resistance [1-3].

The photodynamic therapy (PDT), a novel raw material from plants and multi-herb extractions were developed for the treatment of acne recently [3-7]. Epigallocatechin-3-gallate (EGCG) suppressed the lipogenesis, inhibited the *P. acnes* and inflammation to against acne. Lupeol, a pentacyclic triterpene, identified as a therapeutic agent targeting multiple pathogenic factors of acne. We combined two highthroughput screening methods focus on anti-bacterial and anti-inflammatory to validate lots of active ingredients from different plants, and finally got a multi-herb extraction GMP which consists of the peel of *Garcinia Mangostana*, *Magnolia Officinalis*, and *Punica Granatum*.

In this research, we verified the effect of GMP on treatment for acne, including *P. acnes* suppression, anti-inflammation on human SZ-95 sebocytes and HaCaT keratinocytes, as well as beneficial effects on follicular hyperkeratosis. Further cellular studies indicated that the modulation of TLR2/NF-κB signaling pathways mediated GMP's anti-inflammatory effects on keratinocytes. Therefore, GMP will be used to cure acne focus on multiple pathogenic factors.

Materials and Methods

Microbial Experiments

P. acnes isolate ATCC 11827 was acquired from Beijing zhongkezhijian Biotechnology (Beijing, China). *P. acnes* were cultured on thioglycollate medium. The concentration was adjusted to the 0.5 McFarland turbidity and finally diluted with 100-fold in medium. GMP was originally dissolved in PBS to the concentration of 10% and diluted to 0.01% by serial dilution. Then, 180 µl *P. acnes* with 20 µl samples were added into a 96-well plate, with a 48 hrs co-culture at 37°C in anaerobic chamber. The optical density of *P.acnes* growth was detected at 620 nm on a microplate reader (Multiscan FC; Thermo Fisher Scientific, USA) according to literatures [6,8]. Results between GMP containing solution and vehicle only solution were compared and the growth inhibition at OD620 can be determined from the following equation:

$$\text{Inhibition (\%)} = [1\text{-}OD620_{treatment}/OD620_{control}]\times 100\%$$

For the experiments related with inflammation, *P. acnes* cultures were heat-killed by subjecting to temperatures of 85°C for 30 mins [5,6].

Cell Culture and Stimulation

The SZ-95 immortalized human sebocyte cell line and the human keratinocyte cell line HaCaT were cultured and maintained in DMEM mediumsupplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic solution (5000 U/ml penicillin and 5 mg/ml streptomycin; Invitrogen, CA) at 37°C in a 5% CO_2 incubator as described [4-6].

For stimulation experiments, SZ-95 and HaCaT cells were incubated with the heat-killed *P. acnes* suspension adjusted at the appropriate concentration in culture medium for the desired period of time at 37°C in 5% CO_2 [5,6].

Cell Viability Test

Measurements were performed as described in literatures [9]. In brief, SZ-95 and HaCaT cells were seeded in a 96-well plate at a density of 2 or 1×10^4 cells per well. And then cells were treated with GMP (1% to 0.06% by serial dilution), with or without *P. acnes* stimulation. Medium alone was used as a control. After 24 hrs treatment, 0.5 mg/ml of 3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyl -tetrazolium bromide (MTT; Sigma, MO) was introduced into each well and incubated for another 4 hrs at 37°C. The converted dye was solubilized with DMSO. The optical density of the wells was determined using the Multiscan FC (Thermo Fisher Scientific, USA) at 492 nm. The cell viability at OD492 can be determined from the following equation:

$$\text{Viability (\%)} = OD492_{treatment}/OD492_{control}\times100\%$$

mRNA Expression Measurement in HaCaT Cells

HaCaT cells were seeded in 6-well plates at a density of 3×10^5 per well. After 24 hrs of GMP treatments on HaCaT cell culture, total RNA was extracted by PureLink® Mini Kit (Invitrogen, CA), and the concentration was determined by measuring the A_{260} of the samples. The first-strand cDNA was synthesized from 0.5 µg RNA and real-time polymerase chain reactions (PCRs) were performed according to manuals from the TaqMan® RNA-to-CT™ Kits (Invitrogen, CA). Primer sequences of keratin 16 and 10, loricrin, involucrin, transglutaminase 1, filaggrin and caspase-14 were recorded according to manufacturers' manual of TaqMan® Probe/ROX q-PCR kit (TaqMan® Applied Biosystems, NJ) [10-12]. Real-time PCR were conducted in an ABI 7500 system (Invitrogen, CA) accompanied with CT values.

For the experiment of HaCaT keratinocytes stimulated with heat inactivated *P. acnes*, primers for interleukin (IL) -1α, toll-like receptor 2 (TLR-2) and nuclear factor kappa-B (NF-κB) were also used [13]. The mRNA level of each sample for each gene was normalized to that of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA in all experiments. The relative fold change was quantificated by $2^{-\Delta\Delta CT}$ method compared with untreated controls from the following equation:

$$\Delta\Delta CT = (CT_{\text{target gene}} - CT_{GAPDH})\ \text{treatment} - (CT_{\text{target gene}} - CT_{GAPDH})\ \text{control}$$

mRNA Expression Measurement in SZ-95 Cells

SZ-95 cells were seeded in 6-well plates at a density of 6×10^5 per well, and then stimulated with heat inactivated *P. acnes* and GMP for 24 hrs. The mRNA level of IL-1α was determined as mentioned above.

ELISA Experiments

SZ-95 and HaCaT cells co-cultured with *P. acnes* for 24 hrs were treated with control and GMP (0.1% to 0.01% by serial dilution) similar with mRNA expression assay. Prostaglandin E2 (PGE-2) and IL-6 protein in the supernatants of SZ-95 and HaCaT cells were determined with the corresponding ELISA kit (Neobioscience, China) according to the manufacturers' instructions [14,15]. The concentration of PGE-2 and IL-6 was calculated by standard curves reading at OD450.

Statistical Analysis

All experiments were repeated at least three times with different batches of cells. Data were evaluated statistically using Student's t-test. Statistical significance was set at $P<0.05$.

Results

GMP inhibits the growth of *P. acnes*

To compare dose-response effects of GMP and salicylic acid (SA) on the growth of bacteria that is present in the acne vulgaris, *P. acnes* were co-cultured with either agent at various concentrations for 48 hrs. Bacterial growth was evaluated by measuring absorbance at 620 nm **(Figure 1)**. Both GMP and SA were tested at concentrations of serial dilutions from 1% to 0.001%. We found that when GMP concentration is higher than 0.03%, it killed almost the whole *P. acnes*. In contrast, no killing was observed when GMP is at 0.01%. Therefore, we estimated the minimal inhibitory concentration (MIC), the lowest concentration to prevent bacterial growth of GMP against *P. acnes* (ATCC 11827) was 0.03%. The MIC of SA was 0.3% meanwhile. This suggests that GMP has much stronger antimicrobial activity than SA.

P. acnes (1×10^6 CFU per ml) were incubated with 0.001-1% of GMP (solid circles) and SA (open circles) in medium for 48 hrs under anaerobic conditions at 37°C. After incubation, OD_{620} of each sample was measured by a microplate reader to determine bacterial growth. Data represent mean ± SD of three individual experiments (**P<0.01 by Student's t-test *vs.* control).

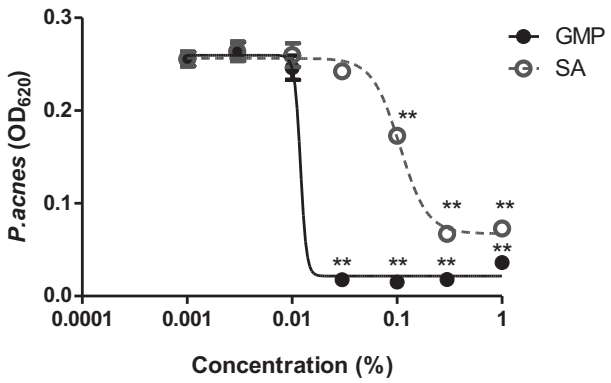


Fig. 1 Bactericidal effects of GMP on *P. acnes*.

GMP promoted the viability of HaCaT cells when stimulated with *P.acnes*

Human sebocyte and keratinocyte are the major target cells of *P. acnes* in acne patients [16,17]. So cytotoxicity of P.acnes and GMP was examined on SZ-95 and HaCaT cells. Firstly, the two cell lines were incubated with GMP at various concentrations from 0.06-1% for 24 hrs at 37°C and MTT assay was used to detect the viability subsequently **(Table 1)**. It was found that GMP did not affect sebocyte and keratinocyte viability at the high concentration of 0.125%, at which *P. acnes* was completely killed as shown in **Figure 1**. And interesting, in HaCaT cells, GMP promoted the cell viability to 121.8% under 0.125% concentration, indicated that GMP may be stimulating the activity of mitochondri to regulate epidermal renewal.

Then, the cytotoxicity of *P. acnes* was tested in these two cells. Results demonstrated that *P. acnes* affected the cell proliferation in HaCaT cells **(Table 2)** but showed little influence in SZ-95 cells (data not shown). The viability of HaCaT cells was decreased to 72.2% when co-cultured with *P. acnes*, and GMP recovered the viability to 108.0% under 0.1% concentration compared with control. This data indicated that GMP can reject the *P. acnes*' effect on cell proliferation to against acne.

Viability %	GMP *Concentration*, %					
	0	0.06	0.125	0.25	0.5	1
SZ-95	100.0±0.6	101.7±1.1	80.0±5.4**	4.5±2.0**	7.4±1.2**	20.7±1.3**
HaCaT	100.0±3.9	128.9±3.9**	121.8±3.0**	45.8±2.8**	3.1±0.4**	1.2±0.3**

*The SZ-95 (2×10^4 cells) and HaCaT (1×10^4 cells) were cocultured with 0.06-1% of GMP for 24 hrs at 37°C. After incubation, cell viability was determined by MTT assay. Data represent mean ± SD of three individual experiments (**P<0.01 by Student's t-test vs. control).*

Tab. 1 Effect of GMP on the viability of cells

Viability %	Control	*P.acnes*				
		GMP *Concentration*, %				
	0	0	0.03	0.1	0.3	1
HaCaT	100.0±2.2	72.2±1.3**	93.4±1.5##	108.0±1.6##	81.2±1.8##	3.6±0.3##

*The HaCaT (1×10^4 cells) were cocultured with 0.03-1% of GMP and heat-killed P. acnes for 24 hrs at 37°C. After incubation, cell viability was determined by MTT assay. Data represent mean ± SD of three individual experiments (**P<0.01 by Student's t-test vs. control; ##P<0.01 by Student's t-test vs. P. acnes).*

Tab. 2 Effect of GMP on the viability of HaCaT cells after *P. acnes* stimulation

GMP decreases inflammation induced by *P. acnes* through the TLR-2 and NF-κB

P. acnes colonization of the follicule is another critical pathological factor for acne, which increases the inflammatory response mainly through the TLR-2 and NF-κB pathways [18, 19]. Heat-inactivated *P. acnes* were recognized by human innate immune system, to induce several molecular markers of inflammation, including IL-1α, IL-1β, tumor necrosis factor (TNF) - α, IL-6, IL-12, PGE-2 and IL-8, in sebocytes, keratinocytes and macrophages [18-20]. We investigated anti-inflammatory effects of GMP in SZ-95 sebocytes **(Figure 2a-b)** and HaCaT keratinocytes **(Figure 2c-e)**, two major cutaneous target cells of acne by ELISA and real-time PCR analysis. Results indicated P.acnes stimulated inflammation by up-regulating the expression of cytokines, such as PGE-2, IL-6, IL-1α and IL-8. When co-cultured with GMP (0.01-0.1%) and *P. acnes*, the secretion of PGE-2 and IL-6 in cells was significantly inhibited, and the mRNA level of IL-1α and IL-8 was also down-regulated, strongly supporting its anti-inflammatory effect.

To further investigate the underlying molecular mechanisms, we checked the activation of NF-κB transcription factor and the mRNA level of TLR-2 responsible for regulation of the inflammatory response **(Figure 2f)** in HaCaT cells. We found that the mRNA expressions of NF-κB p65 and TLR-2 were increased after *P. acnes* treatment,

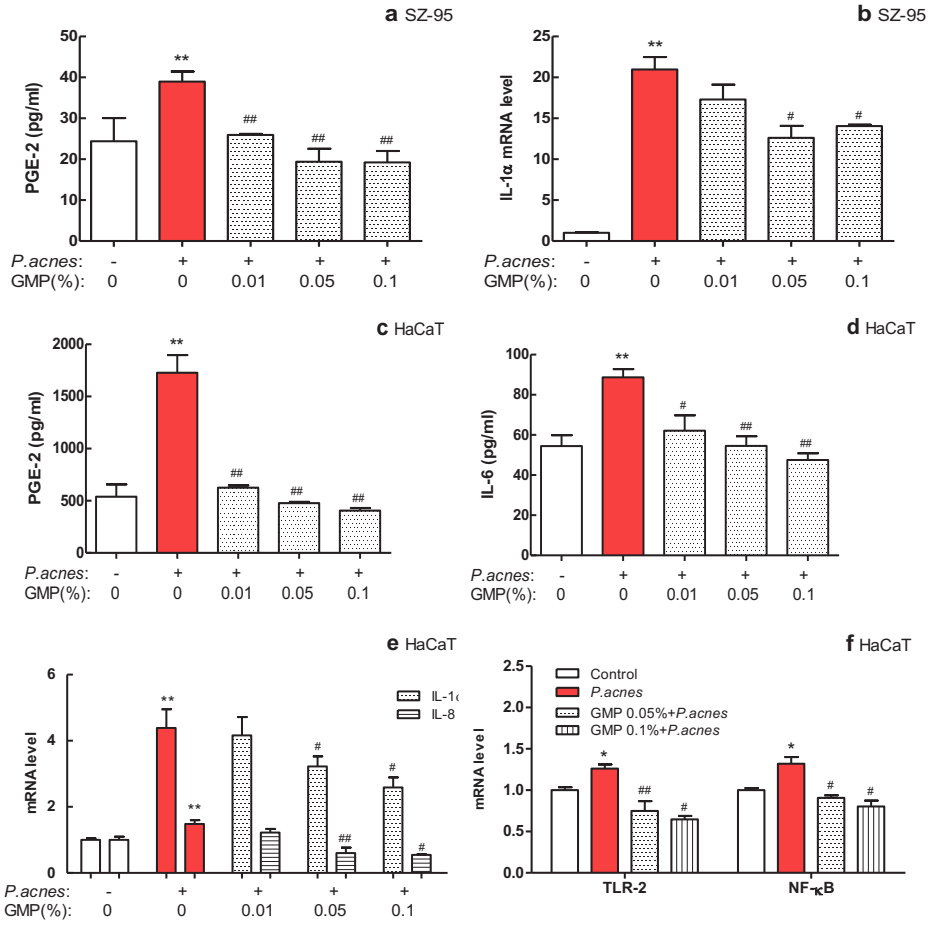


Fig. 2 GMP decreases inflammation induced by *P. acnes* through the inhibition of TLR-2 and NF-κB. (a-f) SZ-95 and HaCaT cells stimulated with *P. acnes* were treated with GMP (0.01%-0.1%) for 24 hours. ELISA for PGE-2 protein (a) in the supernatant and real-time PCR for IL-1α (b) mRNA level in the cultured SZ-95 cells were performed after treatment. ELISA for PGE-2 (c) and IL-6 (d) proteins in the supernatant, real-time PCR for IL-1α and IL-8 (e) mRNA levels in the cultured HaCaT cells were performed after treatment. Then, the TLR-2 and NF-κB mRNA (f) expression in HaCaT cells were also detected using the real-time PCR method. Data represent mean ± SD of three individual experiments (**P<0.01, *P<0.05 by Student's t-test vs. control; ##P<0.01, #P<0.05 by Student's t-test vs. *P. acnes*).

suggesting the activation of inflammation, while GMP significantly down-regulated the mRNA levels under 0.05% to 0.1% concentrations for HaCaT keratinocytes. It's confirming that GMP inhibited innate immunity of two major cutaneous cells associated with inflammatory acne by down-regulating the TLR-2 receptor and mitigating the NF-κB pathway stimulated by heat-inactivated *P. acnes* **(Figure 2a-f)**.

GMP inhibited the mRNA expression levels related to hyper keratinization

Because follicular epidermal dyskeratosis is another major triggering factor of acne pathogenesis, and GMP down-regulated the IL-1α mRNA expression which has been reported to induce hyper keratinization in follicular infundibulum *in vitro* and *in vivo* [21], we investigated the possible beneficial effects of GMP on other genes related to terminal differentiation of epidermal keratinocytes, including keratins K16, K10, transglutaminases TGase 1, loricrin, involucrin, filaggrin and caspase 14 [22,23] **(Table 3)**.

Mediator	Gene	Catalogue
Keratins	K16	Hs00373910_g1
	K10	Hs00166289_m1
Keratinocyte differentiation-specific markers	TGase 1	Hs00165929_m1
	Loricrin	Hs01894962_s1
	Involucrin	Hs00846307_s1
	Filaggrin	Hs00856927_g1
	Caspase 14	Hs00201637_m1
House-keeping protein	GAPDH	Hs02786624_g1

Tab. 3 The catalogue of sense and antisense primer sequences

Discussion

P. acnes, a Gram-positive anaerobic bacterium, is a commensal of human skin, and its overgrowth is closely implicated in the progression of inflammation in acne. Until recently, various antibiotics have been used to control the overgrowth of *P. acnes*, yet increasing antibiotic resistance and biofilm for-

It was found that GMP significantly down-regulated all the genes listed above especially for K10, involucrin and caspase 14 (below to 0.1), showed an inhibited effect on keratinocyte differentiations **(Figure 3a)**.

P. acnes modulate the expression of genes coding keratinocyte proteins implicated in the terminal differentiation of the epidermis, such as transglutaminases, involucrin and filaggrin in a strain dependent manner [22]. We confirmed this result, and also found P.acnes induced the mRNA expression of K16 and caspase 14 **(Figure 3b)**, leading to hyper keratinization in follicular infundibulum. Subsequently, we detected GMP's effect on these five genes after P.acnes stimulated and results demonstrated GMP down-regulated the mRNA expression of K16, TGase 1, involucrin, filaggrin and caspase 14 **(Figure 3b)** which remarkably increased by *P. acnes*, to suppress hyper-keratinization in acne. A schematic diagram of GMP's therapeutic mechanisms based on whole experimental results is illustrated in **Figure 4**.

mation lead to a poor outcome. An accumulating body of evidence suggests that lots of natural active ingredient from plant extracts shows antimicrobial activity against a diverse range of microorganisms, including bacteria, viruses, and fungi. Indeed, we found that GMP, a multi-herb extraction of *Garcinia Mangostana*, *Magnolia Officinalis*, and *Punica Granatum*, significantly inhibits the growth of *P. acnes*. This antibiotic effect of GMP may provide an advantage as a therapeutic strategy for the treatment of acne, especially considering increasing concerns about antibiotic-resistant bacteria.

Many human diseases are often associated with a chronic inflammatory response, which also has a critical role in the development of acne. We found that GMP suppresses the inflammatory response induced by heat-inactivated *P. acnes* in SZ-95 sebocytes and HaCaT keratinocytes, two major well-established in vitro models of inflammatory acne, through the inhibition of TLR-2 and NF-κB pathways. It is also remarkable that GMP decreases IL-1α in HaCaT and SZ-95 cells, based on the fact that IL-1α induces hypercornification of the infundibulum in a manner similar to that seen in comedones. These results suggest that GMP might reverse the altered keratinization of follicular keratinocytes through the regulation of IL-1α.

Anomalies in the infra-infundibulum of proliferation, adhesion and differentiation of keratinocytes lead to the formation of a micro-comedo. The differentiation of keratinocytes also occurs in healthy skin, namely the formation of the cornified cell envelope (CE). This reaction is controlled by various factors. In this study, we investigated the influence of keratin K10 and K16, CE related proteins such as involucrin, TGase-1, loricrin, filaggrin and caspase 14, which are the main component proteins of keratinocyte differentiation. On the other hand, there have been reports demonstrated that filaggrin expression was increased in acne lesions. GMP significantly decreased the mRNA expression of keratins and CE proteins in its own and in heat-inactivated *P. acnes* stimulated manner. Together, these data provide insight to the molecular basis of the therapeutic effects of GMP on the inflammatory acne lesions in a clinical trial.

In summary, GMP modulated the key pathological factors contributing to acne, including *P. acnes* overgrowth, inflammation and hyper keratinization in follicular infundibulum. These results strongly suggest the potential clinical feasibility of GMP in acne treatment.

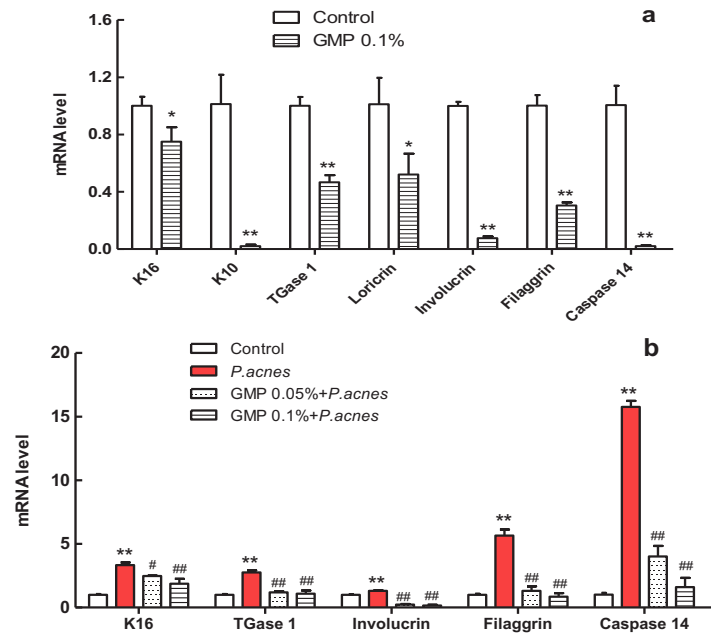


Fig. 3 GMP decreases the mRNA expression related to keratinocyte differentiation on HaCaT cells. HaCaT (1×10^4 cells) were cocultured with 0.1% of GMP and *P. acnes* for 24 hrs at 37°C. After incubation, total RNA was extracted to detect the expression level of genes by qRT-PCR. **(a)** Seven genes were influenced by GMP only. **(b)** Five genes were regulated by GMP after *P. acnes* stimulation. Data represent mean ± SD of three individual experiments (**P<0.01,*P<0.05 by Student's t-test *vs.* control; ##P<0.01,#P<0.05 by Student's t-test *vs. P. acnes*).

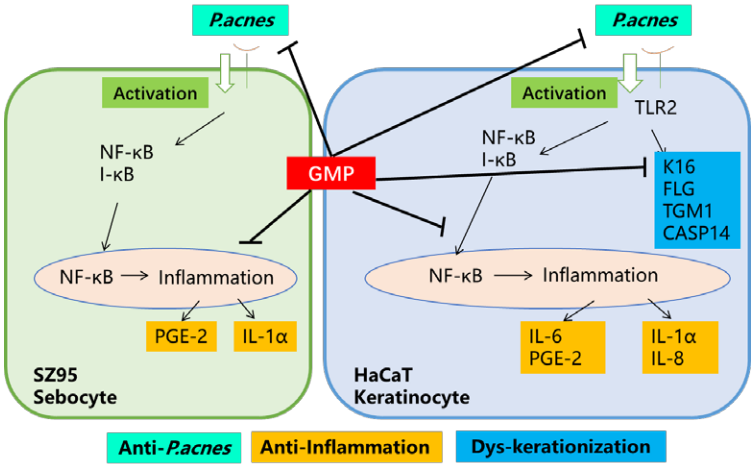


Fig. 4 Possible therapeutic mechanisms based on our *in vitro* results. These mechanisms could elucidate the efficacy

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Conflict of interest

All authors are employees of Shanghai Greaf Biotech Co. Ltd, China. The authors declare no conflict of interest.

Affiliations

Yi-na Lu: study design, *in vitro* experiments, manuscript drafting and revising; Guang-yin Wang and Hong Xie: compound extraction from plants and GMP preparation; Jun Tian: study design and confirm,equipment and financial support; All authors reviewed the manuscripts.

References:

[1] *I Kurokawa, FW Danby, Q Ju, et al.* New developments in our understanding of acne pathogenesis and treatment [J].Experimental Dermatology, 2009, 18 (10): 821-832.

[2] *A Charakida, M Charakida, AC Chu.* Double-blind, randomized, placebo-controlled study of a lotion containing triethyl citrate and ethyl linoleate in the treatment of acne vulgaris [J]. British Journal of Dermatology, 2007, 157 (3): 569-574.

[3] *YH Sun, JI Na, CH Huh and KC Park*. The Effect of Photodynamic Therapy Using Indole-3-Acetic Acid and Green Light on Acne Vulgaris [J]. Annals of Dermatology, 2012, 24(1): 56-60.

[4] *M Im, SY Kim, KC Sohn, et.al.* Epigallocatechin-3-Gallate Suppresses IGF-I-Induced Lipogenesis and Cytokine Expression in SZ95 Sebocytes [J]. Journal of Investigative Dermatology, 2012, 132 (12): 2700-2708.

[5] *YY Ji, HH Kwon, SU Min, et.al.* Epigallocatechin-3-Gallate Improves Acne in Humans by Modulating Intracellular Molecular Targets and Inhibiting P. acnes [J]. Journal of Investigative Dermatology, 2013, 133 (2): 429-440.

[6] *HH Kwon, JY Yoon, SY Park, et.al.* Activity-guided purification identifies lupeol, a pentacyclic triterpene, as a therapeutic agent multiple pathogenic factors of acne [J]. Journal of Investigative Dermatology, 2015, 135 (6): 1491-1500.

[7] *KI Kim, HJ Nam, M Kim, et.al.* Effects of herbal medicine for dysmenorrhea treatment on accompanied acne vulgaris: a study protocol for a randomized controlled trial [J]. Bmc Complementary & Alternative Medicine, 2017, 17 (1): 318-325.

[8] *CH Liu, HY Huang.* In vitro anti-propionibacterium activity by curcumin containing vesicle system [J]. Chemical & Pharmaceutical Bulletin, 2013, 61 (4): 419-425.

[9] *EG Lim, GT Kim, MK Bo, et al.* Study of Anti-microbial Activities and Anti-inflammatory Effects of Chamomile (Matricaria chamomilla) Extracts in HaCaT cells [J]. JKsbb Journal, 2017, 32 (1): 9-15.

[10] *YN Lu, YD Yang, ZY Xie.* Promoting effect of Gmoist Sea-Gel on skin hydration and barrier function [J]. China Surfactant Detergent & Cosmetics, 2017, 47 (12): 709-712. Chinese.

[11] *S Gretherbeck, I Felsner, H Brenden, et al.* Urea uptake enhances barrier function and antimicrobial defense in humans by regulating epidermal gene expression [J]. Journal of Investigative Dermatology, 2012, 132 (6): 1561-1572.

[12] *B Skiba, B Neill, TJ Piva*. Gene expression profiles of TNF-α, TACE, furin, IL-1β and matrilysin in UVA- and UVB-irradiated HaCat cells [J]. Photodermatology Photoimmunology & Photomedicine, 2005, 21 (4): 173.

[13] *TH Tsai, LT Chuang, TJ Lien, et al.* Rosmarinus officinalis extract suppresses Propionibacterium acnes-induced inflammatory responses[J]. Journal of Medicinal Food, 2013, 16 (4): 324.

[14] *M Fritz, AM Klawonn, A Nilsson, et al.* Prostaglandin-dependent modulation of dopaminergic neurotransmission elicits inflammation-induced aversion in mice [J]. Journal of Clinical Investigation, 2016, 126 (2): 695-705.

[15] *M Zeng, ZY Li, J Ma, et al.* Clarithromycin and dexamethasone show similar anti-inflammatory effects on distinct phenotypic chronic rhinosinusitis: an explant model study[J]. BMC immunology, 2015, 16 (1):37.

[16] C *Beylot, N Auffret, F Poli, et al.* Propionibacterium acnes: an update on its role in the pathogenesis of acne [J]. Journal of the European Academy of Dermatology & Venereology, 2013, 28 (3): 271-278.

[17] *U Jappe.* Pathological mechanisms of acne with special emphasis on Propionibacterium acnes and related therapy [J]. Acta Derm Venereol, 2003, 83 (4): 241-248.

[18] *BR Vowels, S Yang, JJ Leyden.* Induction of proinflammatory cytokines by a soluble factor of Propionibacterium acnes: implications for chronic inflammatory acne [J]. Infection and Immunity, 1995, 63 (8): 3158-3165.

[19] *GW Agak, M Qin, J Nobe, et al.* Propionibacterium acnes Induces an IL-17 Response in Acne Vulgaris that Is Regulated by Vitamin A and Vitamin D [J]. Journal of Investigative Dermatology, 2014, 134 (2): 366.

[20] *HH Tsai, WR Lee, PH Wang, et al.* Propionibacterium acnes-induced iNOS and COX-2 protein expression via ROS-dependent NF-kappa B and AP-1 activation in macrophages [J]. Journal of Dermatological Science, 2012, 69 (2): 122-131.

[21] *JL Selway, T Kurczab, T Kealey and K Langlands.* Toll-like receptor 2 activation and comedogenesis: implications for the pathogenesis of acne [J]. BMC Dermatology, 2013, 13 (1): 1-7.

[22] *N Akaza, H Akamatsu, M Kishi, et al.* Effects of Propionibacterium acnes on various mRNA expression levels in normal human epidermal keratinocytes in vitro [J]. Journal of Dermatology, 2009, 36 (4): 213-223.

[23] *E Candi, R Schmidt, G Melino.* The cornified envelope: a model of cell death in the skin [J]. Results & Problems in Cell Differentiation, 1998, 24 (4): 175.

authors

Yi-na Lu, Guang-yin Wang, Jun Tian

Shanghai JAKA Biotech Co. Ltd. | Shanghai | China

Corresponding author: tianjun@greaf.com.cn

The Senior Hair Care Market: Active Ingredients for Healthy Scalp & Hair

A. Momméja

abstract

24 % of the world population is older than 50 years old. In Europe and in the United States of America, these values respectively reach 40% and 36% of the population. How does ageing influence their beauty routine needs? And what does it mean for hair care? 24% of the world population is older than 50 years old. In Europe and in the United States of America, these values respectively reach 40 % and 36 % of the population [1]. Seniors born between 1946 and 1964, the last year in which annual births exceeded 4 millions, are called baby-boomers and belong to the "Silver generation". They grew up in a consumerism and marketing boom, evolving in a positive economic context [2]. How does ageing influence their beauty routine needs?

Introduction

If, on one hand, 67% of the 55 and over accept having a longer beauty routine to answer the new needs of their skin and hair, on the other hand 67% of boomer women like to experiment new products to feel confident [2], highlighting the importance of beauty care to address these consumers and empower them. In the end, the first step to consumer satisfaction is to show understanding for their needs and to offer dedicated care. But what does it mean for hair care?

The industry focused a lot in the past decades on hair colorations as the main hair concern of seniors. In the past years though, the well-ageing and inclusivity movements showed a growing tendency [4]. Grey or white hair are not meant to be covered anymore, but to be proudly assumed as a way to empowerment and self-confidence. Consumers want to age well and to have "healthy" hair, whatever is the color. Grey hair has partly hidden the other concerns of consumers so far. And indeed, age influences hair in many other ways than just color, especially for women.

With the menopause, the synthesis of collagen decreases, the lipid production in skin and scalp decreases and skin dryness increases. The metabolism slows down and consequently, the epidermal renewal is less efficient overtime [3]. Hair growth may become slower and less performant, up to hair loss, and hair may also lose its pigmentation. All these factors impact the scalp and the hair growing on it, becoming thinner and lacking volume. These parameters were identified by Mintel like the concerns to address specifically when targeting the senior market(4). "Wesource by seppic" developed different solutions adapted to the senior hair care market: a sea beet extract, targeting damaged hair, hair volume and cell senescence, a golden samphire extract, targeting hair protection, energizing and lipid replenishing effects and a 3-in-1 tonic, rich in vitamin, to boost hair growth and density.

A sea beet extract for smooth hair and volume

The sea beet is a halophytic plant, leaving on the seaside and resistant to different stresses like salt, UV and wind. This environment confers it an interesting composition in antioxidant molecules. Our oil soluble extract was tested *in-vitro* for scalp benefits and *in-vivo* for hair fiber benefits. *In-vitro*, on reconstituted epidermis, it demonstrated a significant reduction of lipoperoxidation (-13%) versus non treated, thus reducing the risk of oxidative damages on scalp and hair fiber. Tested on keratinocytes exposed to UVB stress, it also exhibited significant soothing action from 0,2% by reducing the amount of inflammatory mediators like PGE2 (-13%), IL-1 (-21%) and IL-6 (-21%). Finally, it was shown that the sea beet extract protects the cell from ageing by significantly slowing down the senescence process (beta-galactosidase assay).

In-vivo, the sea beet extract was tested at 1% on 20 women with dry & damaged hair. The structure of the hair fibers was evaluated by an electron microscope. After 28 days of daily use, the hair structure was significantly improved versus D0: +62% improvement of scales aspect (smoothing effect). The self-evaluation also allowed identifying the key benefits perceived by the consumer. Shine improvement was the benefit n° 1 with +22% satisfaction versus placebo while volume was the benefit n° 2 with +14 % satisfaction versus placebo.

This global approach, combining scalp and hair benefits, is extremely interesting for seniors exposed to increased scalp and hair dryness and inflamm'ageing.

A golden samphire extract for hair protection & lipid replenishment

In the same way as the sea beet, the golden samphire is a halophytic plant having a stress resistant profile. Our oil soluble golden samphire extract was tested *in-vitro* for scalp benefits and on tresses for hair fiber benefits. Tested on re-constituted epidermis, under physiological and under UVB stress conditions, it demonstrated a significant energizing action by 14 and 22% respectively by measuring the cell mitotic index. Furthermore, tested on explants, it demonstrated a significant boosting effect of total lipids (+73%) and also more particularly polar lipids (+87%), both categories, polar and apolar, playing an important role in the internal and external hair lipidic barrier **(Figure 1)**.

On tresses, the golden samphire extract was tested at 1% in a rinse-off application. After a single shampoo, an increase in shine and softness was observed, thus making the product of interest for dull and damaged hair.

Our golden samphire extract, with replenishment of dry scalp and hair benefits, is therefore very well suited to fulfill senior's hair & scalp needs. Energizing benefits complete these effects by supporting a better cell renewal balance and preventing metabolism slow-down.

A mixture of pro-vitamin B5 and seaweed extracts for higher hair density

Enriched in pro-vitamin B5, our 3-in-1 tonic also contains two seaweed extracts: an extract from the channeled wrack, a seaweed with high resistance to desiccation and rich in antioxidant molecules, and an oarweed extract, known for

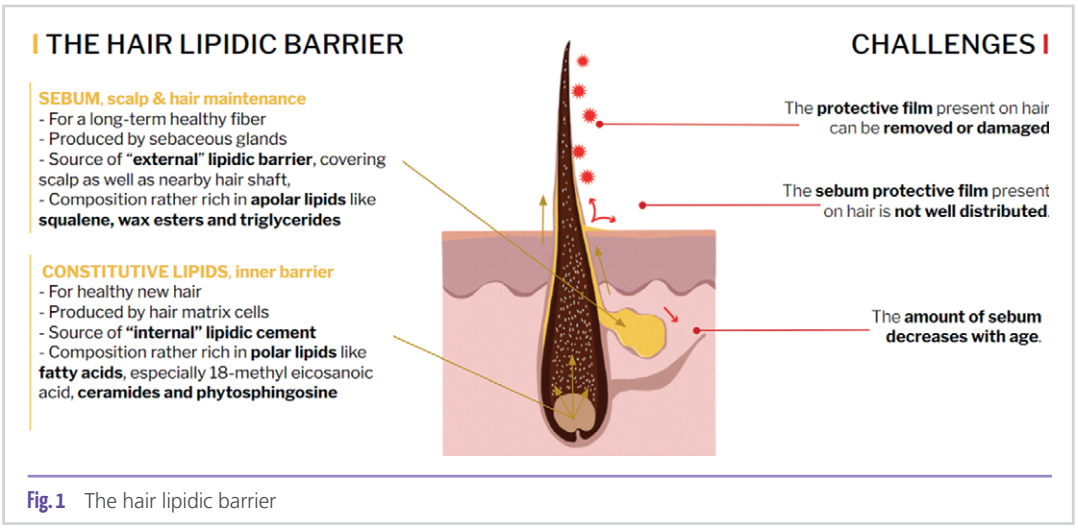

Fig. 1 The hair lipidic barrier

its nutritional value and traditionally used as a fertilizer. We tested this mixture *in-vivo* on volunteers with a chronical hair loss. We compared the growth speed and the hair density at D3 and D63 after shaving **(Figure 2)**.

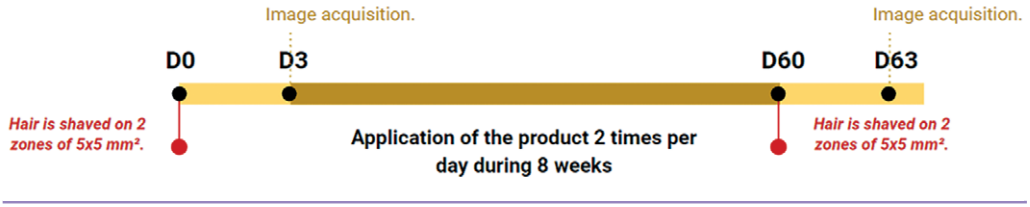

Fig. 2 *In-vivo* protocol of the 3-in-1 tonic (pro-vitamin B5 + seaweed extracts)

After 8 weeks of treatment, we observe an increase of +27% hair length and +10% hair density **(Figure 3)**. Even without any application from D60 to D63, hair is able to grow faster and in higher density versus before treatment, translating a long-term scalp condition improvement. Thus, this study highlights the long-term scalp healthiness benefits, being often underestimated and neglected.

Conclusion

The senior hair care market is evolving. First, the target population is bigger than ever. Secondly, the progressive empowerment of generations has been leading to less complexes and shame towards ageing, thus changing consumers' expectations. The focus is not on "hiding ageing" anymore, but rather on "exhibiting well-ageing". A new category of ingredients deserves therefore to be highlighted to address these new consumers. Our two oily extracts from the sea beet and the golden samphire are particularly of interest to replenish hair and scalp in cleansing routines as well as in daily care products. Our 3-in-1 tonic is of interest for nourishing and stimulating scalp health and ensuring healthy hair growth. Indeed, healthy scalp and hair are the key claims to hair care well-ageing.

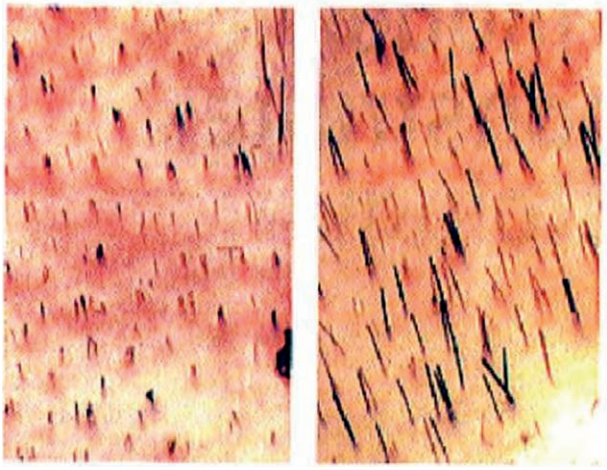

Fig. 3 Scalp at D3 on the left, Scalp at D63 on the right

References:

[1] https://population.un.org/wpp/DataQuery/

[2] The Senior BPC Consumer - UK, Mintel, 2018

[3] *H Bensaleh, F Z Belgnaoui, L Douira, L Berbiche, K Senouci, B Hassam;* Skin and Menopause - 2006, Dec

[4] Mintel, Silver seniors will future-proof haircare sales

author

Anna Momméja
Hair care active ingredients manager | Wesource by Seppic

Seppic SA | Paris La Défense, 50 boulevard National CS 90020
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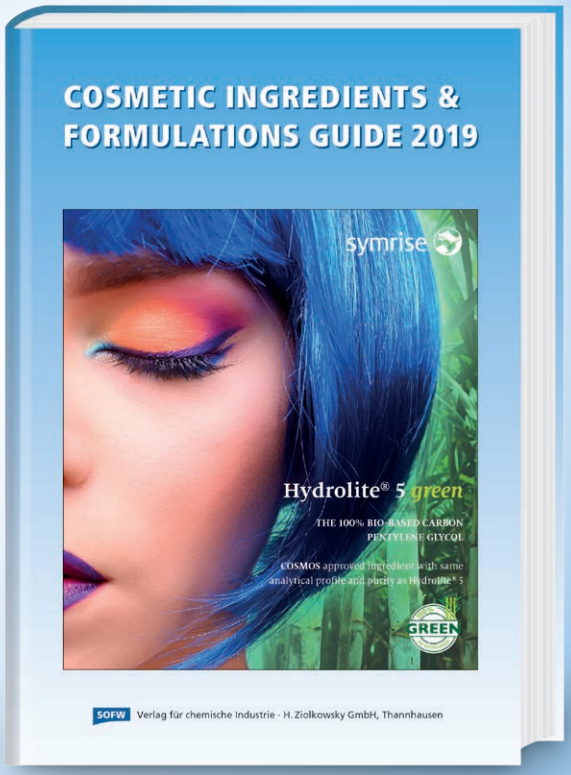

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Microbiological Quality Management for the Control of Quality Costs (Part 1)

J. Nussbaum

abstract

The definition and implementation of measures to ensure microbiological product stability, the success controls for the established measures, the detection of internal and external failures as well as their corrections cause costs, the so-called quality costs. Cosmetic companies are interested in minimizing these costs during the production of cosmetics.
The aim of this document is to present a concept for a possible cost-saving potential for microbiological expenses in the development and production of cosmetics without compromising the product safety.
Every cost-saving consideration is first preceded by an analysis of the failure potentials that could lead to a negative impact on the end product. In every process - whether in product planning, product development or manufacturing of cosmetics - a multitude of such potential failure opportunities can be identified, which are evaluated according to the probability that they will lead to a significant reduction in the quality of the end product. Corresponding protective measures have to be defined and implemented.
The next step is to consider the potential for saving testing costs and to implement cost-optimized measures. It is obvious that such optimization measures should be defined at the earliest when it can be demonstrated over a longer period of time that the entire process regarding quality planning, development and manufacturing leads to consistently good results with regard to microbiological purity and stability of the finished product. Optimization measures should be defined with a sense of proportion and evaluated for suitability by considering the entire process.

1. Introduction

The aim of this document is to present a concept for possible cost-saving potentials for microbiological expenses in the development and production of cosmetics without compromising the product safety. The following are considered:

- Basic considerations on quality costs related to microbiological quality assurance and classification of quality measures to the types of costs (prevention costs, testing costs and consequential failure costs).
- Detection and avoidance of potential failures in product development and product realization (raw materials, formulation, manufacturing specification, manufacturing process, quality of production facilities (hygienic design) and testing equipment, GMP requirements incl. R&D measures, hygiene monitoring, staff training and documentation) to reduce consequential failure costs.
- Cost-saving potentials based on defined, assured process workflows and the application of state of the art methods.

Every cost-saving consideration is first preceded by an analysis of the potential failure opportunities that could lead to a detrimental effect on the finished product. In every process - whether in product planning, product development or manufacturing of cosmetics - a multitude of potential failure opportunities can be identified (e.g. quality of raw materials, quality of water, formulation assurance, manufacturing specification, compliance to GMP, efficiency of R&D measures, hygienic plant design, etc.). These must be evaluated according to the probability that an identified failure will lead to a significant reduction in the quality of the finished product. Corresponding protective measures must also be defined and implemented.

Cost-saving potentials based on defined, assured process workflows and the application of state-of-the-art methods. Modifications in existing processes that are intended to reduce quality costs must be carried out with a sense of proportion. In case of unexpected failures, the previously assured process workflow must be restored immediately. For example, transferring the incoming raw material control to the supplier according to previously agreed methods is a possible optimization of the process. The raw material release is carried out based on the supplied certificates. If a product contamination is caused by a contaminated incoming raw material, the relationship of trust between manufacturer and supplier is disturbed and internal incoming raw material controls have to be carried out again.

In the following, the systematic approach for identifying potential failures and cost-saving is presented. Practical examples show possible saving potentials.

2. Quality costs – General considerations

There are various models for quality costs and each of them represents a slightly differentiated approach and basic statements.

a) Activity-based model (according to J.M. Juran)

This most commonly used model assumes that a balance between disadvantages due to quality costs and advantages by the increasing income can be achieved. For this purpose, the trilogy of "quality planning, quality assurance and quality improvement" is to be used. In the course of the process, it is important to control the three types of costs mentioned below (see also 2.1), since the total quality costs strongly depend on their magnitude and temporal frequency.

- Prevention costs
- Testing costs
- Consequential failure costs

b) Impact-oriented model

In this newer model, the quality costs are assigned to their task:

- Conformity costs
- Non-conformity costs

While the costs required to reliably obtain the defined quality are summarized as conformity costs (i.e. prevention costs and testing costs according to point a), non-conformity costs derive from the non-fulfilment of the quality requirements as additional improvement measures (defect costs and testing costs that become additionally necessary according to a).

c) Failure costs „rule of ten"

A look at the failure cost "rule of ten" shows that the further an failure proceeds undetected into the late stages of a process or comes to light first at the customer's site, the higher are the costs to correct this failure. The costs of an undetected failure increases by a factor of 10 from stage to stage of the value added chain. The earlier failure detection occurs, the more cost-effective it is for the organization **(Figure 1)**.

"The more efficient the procedures and methods in the context of failure prevention and therefore quality improvement, the higher and more cost-effective is the required quality level of a company." (*)

(*) *Omachonu, V.K., Suthummanon, S. and N.G. Einspruch* (2004): The relationship between quality and quality cost for a manufacturing company; in: International Journal of Quality and Reliability Management, Vol. 21 No. 3, S. 277 – 290

2.1 Definition of failure costs according to DIN 55350 and their application to microbiological quality assurance

Classification of failure costs according to DIN 55350 applied to the microbiological quality assurance of cosmetics:

2.1.1 Prevention costs

Costs caused by the analysis and elimination of failure causes

For the systematic analysis of process workflows in microbiological quality assurance, the application of failure prevention analyses (e.g. FMEA, HACCP or a hazard analysis and risk assessment according to the IFS-HPC standard) can be helpful. The analysis is based on the compilation of the individual process or procedure steps, the identification of failures, the evaluation of the significance of the failure for the finished product and the definition of measures to eliminate the potential failure. With the help of such an analysis, existing quality assurance measures can be revised and corrected.

It is also worthwhile to carry out a failure prevention analysis for microbiologically highly sensitive and / or high-priced products. The result leads to a catalogue of measures for the entire process, correct procedures and test plans can be prepared.

Prevention costs originate from microbiological quality assurance. For example, define the following measures for the prevention / elimination of failure causes:

- Quality and test planning for:
  - Product development and scale up phase
  - Raw materials, packaging materials, incoming goods control, storage, risk-based dynamization of incoming goods control, release system
  - Hygiene measures
    - Personal hygiene / personal behavior, training
    - Industrial hygiene in production: hygiene plan / defined cleaning regulations (e.g. R&D in the development of new formulations, consider structural deficiencies in plant design)
  - Product manufacturing and intermediate products: In-process testing, product / finished product testing, release processes, procedures in case of positive results

- Technical optimization:
  - Production facilities (hygienic design): microbiological considerations in planning / expansion
  - Water system: system design / test design
  - Preventive service plan
  - Computer-assisted weighing systems to avoid weighing failures and mix-ups

- Establishment of a documentation system
- Training / Employee motivation
- Supplier assessments
- Internal audits

2.1.2 Testing costs

Costs due to scheduled tests, which are not caused by a specific failure, but which are intended to avoid consequential failure costs

In the microbiological quality assurance of cosmetics, testing costs arise in the following cases:

- Microbiological tests and assessments as part of product development and the scale-up phase,
- incoming goods / storage controls of raw materials and packaging materials,
- Process water monitoring
- Process analyses, in-process controls, end product controls in the manufacturing process
- Hygiene monitoring,
- Market analyses, complaint management.

2.1.3 Failure costs / consequential failure costs

Failure costs are costs caused by a failure

- Internally occurring failures are detected and eliminated before the product is delivered
- Externally occurring failures detected through complaints or market analysis.

This can result in extremely high consequential failure costs. Such costs come from:

- Processing costs for contaminated batches
- Causal research and corrective measures to avoid a repetition of failures.

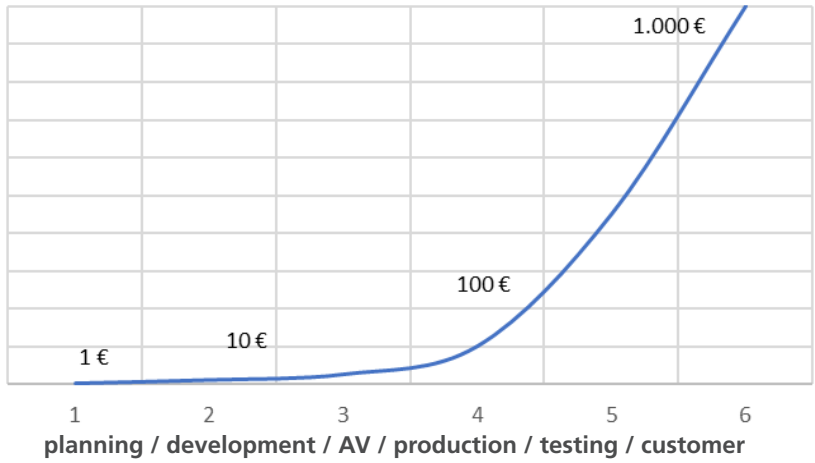

Fig. 1 Prevention costs according to different stages (schematic diagram)

- Production losses
- Post treatment / destruction costs of contaminated goods
- Contractual penalties for non-compliance with delivery dates
- Recall / Image loss
- Additional expenses for extensive R&D measures

3. Systematic avoidance of failure costs

For systematical avoidance of (consequential) failure costs, it is advisable to prepare a very detailed analysis and planning for the entire development and manufacturing process (see 2.1.1). In this context, identify potential sources of failures, if necessary, evaluated by means of risk priority numbers, which are composed as follows:

Probability of occurrence of a defect X / importance of the defect for the finished product Y / probability this defect be noticed. FMEA, HACCP or hazard analysis and risk assessment according to IFS-HPC standard often used here.
Systematic failure analysis leads to a global observation of the system. Single measures out of context always run the risk of creating deficiencies in the protection of other process steps, which can easily be overlooked.

During the systematic failure analysis, in situ observations of the manufacturing process take place. Among other things, observation of parameters set by the development and production documents, formulations and documents for microbiological validation.

The following elements are essential:

- Assessment of raw materials for microbiological vulnerability, specifications and other supplier agreements
- Specifications for production water / Conformity with specifications / Test plans, sampling plans, methods
- Process instruction (VA) on the process starting with the incoming goods inspection until release, for documentation, sampling, for test plans and methods according to the current state of the art (the detection of microorganisms in small numbers in the sample must be ensured).
- Production specification, documents from the scale-up phase, securing of microbiologically relevant process phases and individual process parameters such as pH value, temperature, solubility, etc., securing of water-based raw material pre-solutions, comparison with routine process.

Described and implemented procedures exist for

- Process control, documentation
- Hygiene monitoring
- cleaning and disinfection measures
- Microbiological in-process testing, test plans
- End product release (sampling plan, reserve samples, analyses, methods, specifications)
- Handling of non-conforming end products, blocking, failure analysis
- Procedure for treatment or destruction of contaminated products, assessment of marketability
- Recall from the market
- Dealing with suppliers, customers, contract manufacturers
  - Critical process workflows, quality agreements, etc.

Careful planning and the systematic incorporation of empirical data enable the permanent avoidance of failure costs. Continuous process improvement (CIP) is part of every quality assurance. However, the corresponding planning and implementation also require time, which must be made available.

Examples for Continuous process improvement (CIP):

- Procurement management:
  - Raw material/packaging: Finding of the optimal balance between costs and quality, making concrete agreements with suppliers in advance.
  - Do not simply compare offers - modifications may also require additional testing.

- Scale up: ensure that no failures are generated when transferring a development formulation to production scale.

- Change control (internal changes to existing specifications - ensure that possible effects on other processes/parts of the process are considered).
  - For raw materials/packaging materials/formulations (scale up) depending on the process and production method (e.g. conversion to continuous production)
  - For Personnel (e.g. systematic training, also for temporary staff)
  - For equipment (new units or also repairs)

- How are microbiological analyses designed?
  - Identification in case of growth, testing if reproduction is possible; in case of deviations, modify the procedure according to the results.
  - Allowing early separation of affected batches due to the results of analyses.
  - Allowing early initiation of cleaning and disinfection measures.
  - Updating of process instructions for compliant and non-compliant finished products, if necessary.
  - Trend analyses (routine/monitoring)

- Open communication
  - Ensure interface communication, each sector is contributing to the overall result. If necessary, consider external partners.

Based on the measures determined for the avoidance of potential failure modes (failure prevention costs) and derived process instructions and tests (testing costs), a concept is created that helps to avoid later failures and subsequent failure costs. The PDCA Circle (plan, do, check, and act) can be used for this implementation:

4. Limitation of failure costs (in case of unavoidable failures)

When failures occur, the rule is to detect them as soon as possible in order to avoid an increase in failure costs (see rule of ten). Here, "simple mechanisms" can have a very positive effect if they are supported in a company. It is one of the keys to high efficiency.

In this context, it should be mentioned that regular employee motivation provides immediate reactions. The relationship of trust between supervisors and employees is an important requirement for this

- Employees have the confidence to report deviations
- Reporting chains are known and defined so that reports are always sent to the right decision-making authority.

A concrete example:

During bulk production, a plastic paddle falls into the production vessel.

- **Case 1**: The employee reports this immediately, there is no need to investigate the cause. The damage can be quickly localized.
- **Case 2**: If the employee does not report this immediately, in extreme cases the failure may only be detected by a customer complaint (foreign object in the product) and lead to a recall.

To be continued

In the 2nd part of the article, which will be published in the SOFW Journal December 2021, possible savings in terms of quality costs, based on some practical examples, will be looked at in more detail:

- Quality costs – potential savings
- General considerations
- Determining savings potentials
- Optimization of testing costs / monitoring
- Optimization of the Cleaning costs

authors

For the
DGK expert group "Microbiology and Industrial Hygiene"

Joelle Nussbaum |
BAV Institut GmbH | Offenburg | Germany
Joelle.nussbaum@bav-institut.de

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VINCENTZ

Unsupervised Machine Learning Methods to Understand Relaxation Effect of Fragrances through EEG Technique

C. Mhaskar, V. Kaushik, A. Arjula

abstract

Objective: The objective was :**1)** To validate relaxation effects of fragrances proposed in the previous work using Unsupervised Machine Learning methods (USL). **2)** To compare and quantify the relaxation potential of fragrances by establishing a criterion (metric).
Methods: K-Means and Principal Component Analysis (PCA) were the employed USL methods. The data was a result of administering 4 essential oil fragrances (ALO (control), ECO, Lavender and AROMA) to 50 participants. PCA aided the characterisation of fragrances as function of 8 waves based on the relaxation induced.
Results: The result from the previous work i.e. aromatherapy induced higher relaxation and, Alpha waves were good indicators of relaxation, was validated by K-Means. Higher Alpha wave intensity was associated with fragrance administration. Principal waves for olfactory stimulation were identified as Alpha, Beta and Theta. PCA analysis showed AROMA and Lavender fragrances had higher relaxation potential compared to the other fragrances. Weighted PCA showed the difference in the degree of relaxation for the administered fragrances.
Conclusion: We concluded that Aromatherapy fragrance (a synergistic blend of relaxing essential oils) resulted in higher relaxed mental states. This was achieved by employing USL techniques as comparison and validation metrics for the previous study. Also, USL techniques were used to propose a methodology to understand and characterise EEG sensory data variability (subject-to-subject variability). The Relaxation Potential Metric successfully compared the degree of relaxation induced by olfactory stimuli. The significance of the proposed methodology is :**1)** It can be used as tool to analyse brain wave data from sensory stimulus/stimuli for better comparison and characterisation and **2)** It can be employed to engineer products which are more consumer-centric in nature.

Introduction

We perceive and understand the external world via our five senses. Stimulus to one or more senses creates experiences and aids in learning. This sensory stimulus can either be voluntary or involuntary. For example, hearing the noise around you while seated in a park is involuntary but listening to music is voluntary. For majority of the populace there are inherent patterns and metrics linked with sensory stimulus which, are defined as good/pleasant and bad/ unpleasant experience. For visual stimulus, warm colours like red and yellow induce excitement, increased breathing, and heart rate [1]. And cool colours like blue and green induce concentration, relaxation, joy, slows down heart rate and is soothing for eyes [1]. For sensory stimulus of touch and taste, the experiences are very relative and are a function of environment and culture. For example, the sense of taste is regional in nature where the cuisine in the Asian continent tends to include more spices than its culinary counterparts. Except the sense of smell, all the sensory stimuli are tangible and can be measured.

The sense of smell gives information about the environment through intangible, invisible, and/or inaudible objects and, has a highly reminiscent quality [2,3]. Olfaction entails the odour chemical or odorant entering the nasal cavity and binding to the olfactory receptor. The receptor signals received by the sensory neurons are identified and characterized based on memory and emotions evoked, which affect the mind and body [4,5,6]. This attribute of smell/odour has been leveraged since time immemorial to alter emotions and, the state of one's mind. The earth's macrocosm possessing the ability of smell has evolved and developed responses(mental and physical) for odours like relaxation, fight-or flight, stress, grief, trust, and even increased healing. Amongst all odours available, aromatic plant extracts or essential oils have been used for increased health and well-being, essentially to improve the health of body, mind, and spirit. This holistic healing science has been termed as Aromatherapy [2]. Aromatherapy or essential oil therapy exploits the medicinal nature of the essentials oil or blend of essential oils through its application to skin, oral (vapour inhalation) or olfactory stimulus.

Blended essentials oils in topological applications have shown positive changes in Autonomic parameters (Heart rate, Blood pressure, Pupil functions, Electrodermal activity, Electroencephalogram (EEG)) and Emotional parameters (alertness, vigour, calmness and relaxation) [7]. Olfactory stimulus of certain essential oils like lavender, bergamot, cedarwood and rose otto relieve emotional stress, symptoms of depression and anxiety [8]. Stress and anxiety producing stimuli, increase heart rate whereas relaxing stimulus stabilize or reduce the heart-rate. Fragrances which increase heart rate are called stimulating

aromas (sweet orange oil, valeric acid) and sedative/relaxing aromas decrease heart rate (sweet fennel oil, spiced apple, rose oil). Blood pressure is another physiological variable and also an index of cardiovascular wellness. Blood pressure is a factor of blood volume, peripheral resistance, and stroke volume. Lower blood volume means lower blood pressure and lowering blood pressure indicates relaxed state. Aromas which lower blood pressure are termed as vasodepressors like nutmeg-oil, valerian oil, mace extract and neroli oil. The effect of aroma on the brain wave amplitude and frequency are measured using an EEG. The brain waves can be further characterised based on their frequencies. Alpha waves are the dominant waves during the period of relaxation or mentally relaxed states. Aromas like lavender, bergamot, eucalyptus, spiced apple, cineol, jasmine and alpha-pinene induce a state of relaxation [9].

Signal	Frequency (Hz)	State of Mind
Delta	0.5-3.5	Deep sleep
Theta	4-8	Drowsy, REM sleep
Slow-Alpha	8-11	Relaxation, Eyes closed
Fast Alpha	11-13	Calm, Resting, Idle
Low Beta	13-15	Fast Idle, musing
Mid Beta	15-20	Active thinking, focused, high alert
High Beta	20-30	Complex thoughts, integrating new experience
Gamma	25-42	Mindful, Meditative

Table 1: Table showing mental states characterized by brain wave signals and respective frequency [11,12,13].

EEG and its significance for mental state

Electroencephalogram or EEG is a technique which records spontaneous electrical activity of the brain as a function of time. This is generally a non-invasive electrophysiological technique, which is performed using multiple electrodes strategically placed on the scalp. The most common use of EEG is to diagnose epilepsy, where the brain activity is mapped for any variations in EEG signals. It is also used to detect dementias, coma, anaesthetic patterns, neonatal development, infant and paediatric development [10].

EEG signals are characterized based on their frequencies given in **Table 1**.

Each wave is characteristic of a particular mental state and action. It is seen that Alpha waves are predominantly present when the mental state is relaxed and calm. This relaxed state can be generated using sensory stimuli- Visual, Olfactory, Tactile, Gustatory and Auditory.

Various studies have validated the relation between relaxed mental state and alpha wave change. *Belkofer et al* [14] has tested the effect of motor-visual stimulus on Alpha wave activity using Art therapy. The subjects showed relaxed states post-Art therapy EEG from the Alpha band analysis. The alpha wave change due to visual stimulus was a regulatory behaviour which was built-in through reinforcement aka people with artistic talent. *Phneah et al* [15] demonstrated an Alpha neurofeedback training for mood enhancement using artifact free-EEG. The subjects were fed audio signals (soothing music). Post-stimulus EEG showed higher levels of alpha waves for experiments of varied time. ANOVA results showed statistically significant difference in the mean absolute power pre- and post-stimulus. *Sowndhararajan et al* [16] inspected the effect of fragrances and odour on Human Psychophysiological activity. Aromatic compounds found in essential oils showed increased alpha wave intensity and density with relaxed and calm mental states attributes. Fragrances/odours exclusively contributing to alpha wave increase were m-Xylene, bangalol, white sapphire, indole, linalyl acetate, 5-α-Androstan-3-one, eucalyptus oil, ammonia, valeric acid, soyabean aroma, neroli, grapefruit oil, rose oil, lavender oil, Zizyphus jujuba seed oil and bergamot.

Previous Study and Inspiration for Present Work

The objective of the previous work by *Kaushik et al* [17] was the quantification of olfactory stimulus using psychophysiological markers of relaxation state: quantitatively (EEG) and qualitatively (consumer ratings for relaxation). A protocol was designed around inducing and quantifying stress, olfactory stimulus, control stimulus and relaxed state. The stress test for the protocol was a timed-mathematical problem-solving test. The primary evaluation parameter was the Alpha wave Intensity – a quantification metric for stressed/relaxed mental states. The olfactory stimuli involved 3 essential oil samples (Edible Coconut Oil (ECO), Lavender Oil (Lavender), Synergistic blend of Edible Coconut Oil and essential oils- Bergamot, Rosemary and Lavender oil with Edible Coconut Oil (AROMA)) and 1 control sample (Aroma-less oil sample (ALO)).

50 subjects (24 males and 26 females) participated in the study in the age group of 25-40. The olfactory stimuli were administered under controlled environmental conditions and EEG brain waves pattern was recorded using Neuroelectric "ENOBIO 8". Heart rate was monitored using Scosche's 'RHYTHM+' monitoring band.

EEG signals obtained were pre-processed using Independent Component Analysis (ICA) and Wavelet Decomposition. ICA was performed for artifact removal through blind source separation. It separated multi-source signal into a signal having mutually independent components. Wavelet decomposition

was performed to classify the EEG signal into 8 frequency bands as defined in **Table 1** (Delta, Theta, Alpha, Beta, Low-Beta, Mid-Beta, High-Beta and Gamma). The relaxation potential for the Olfactory stimuli were analysed using Supervised Learning techniques- comparing the alpha wave intensity change from the rest state for each subject. The study concluded that alpha waves decreased under stressed state (timed math test) by ~5% from their rest state level. The alpha waves recovered substantially when exposed to olfactive samples; AROMA exposure showed the maximum benefit while ALO did not show any recovery.

In the analysis, only alpha wave intensity was used to elucidate the relaxation potential of olfactive stimuli and the changes in the other waves were not considered in the analysis. Furthermore, the difference in percent change in alpha wave intensity amongst the three olfactive samples was not high and significance could not be ascertained due the sample size. However, the difference seemed a little subdued as the olfactive samples represented a well-known essential oil for relaxation – Lavender Oil as well as a known synergistic blend (AROMA).

Alpha waves were obviously the main indicative metric for relaxation, but not the only brain waves that underwent a change. *Field et al* [18] investigated the changes in Delta, Theta, Alpha and Beta waves for the relaxation effects of Lavender floral blend cleansing gel. EEG activity assessment of Alpha and Beta waves was done by *Diego et al* [19] (Lavender), to understand the relaxation effects of Aromatherapy. *Lorig and Schwartz et al* [20,21] investigated the effects of spiced apple, eucalyptus and Lavender on Alpha, Beta and Theta wave changes to better understand neural activity. *Klemm et al* [22] investigated the effect of multiple fragrances (Lavender, lemon, and peppermint) to understand changes in the Topographical brain activity (Alpha, Beta, Delta, and Theta). It was seen that Alpha, Beta and Theta waves always suffered changes for Lavender use. Thus, there exists a more complex linkage between different frequency waves induced by a fragrance and the corresponding relaxation potential. In the present work we have used sophisticated data analysis techniques for a better discrimination of the fragrance potential to alter the brain relaxation state.

The objective of the present study was two-fold: 1) Explore Unsupervised Learning algorithms for an improved discrimination between the brain waves signals for the data gathered in previous study, 2) Identifying an improved relaxation metric as a function of all the brain waves for characterising the olfactory stimulated EEG data.

Unsupervised Learning Methods

Unsupervised Learning (USL) is a machine learning algorithm where the statistical structure of an overall collection of input patterns of a system is expressed through specific input patterns. In layman terms, the algorithm draws inferences and recognises patterns in a dataset without any labels. Such algorithms are employed to find features in an unlabelled dataset for characterization. This characterization can be achieved through clustering. Here a cluster (from the entire dataset) is a collection of datapoints which bears some likeliness or resemblance, which is defined based on the approach used. The USL algorithms can be further categorised into Clustering methods, Anomaly Detection methods, Neural Network methods and Latent variable models. A brief introduction regarding the aforementioned sub-categories can be found in the **Appendix**.

Clustering algorithms

Clustering is a machine learning technique, which intends to find a pattern/structure in a collection of unlabelled data. The grouping of data is done using similarity metrics/dissimilarity metric based on the inherent intrinsic pattern of the data. The criterion for cluster separation is very domain specific. It needs to be defined to fit the problem objective. **Figure 1** [23,24,25,26,27] classifies the clustering algorithms for better characterization and understanding as follows:

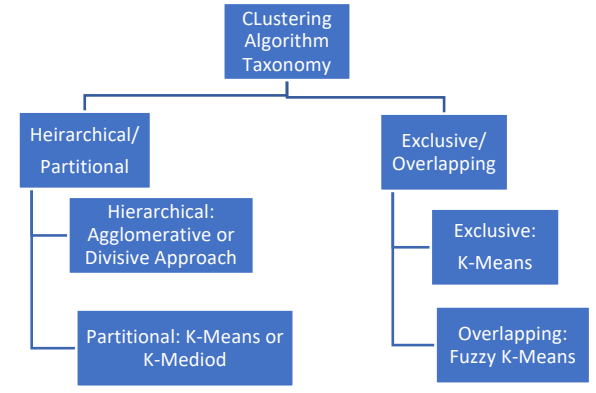

Fig. 1 Figure showing the Taxonomy for Clustering algorithms as proposed by *Kononeko and Kukar et al*

The application of Clustering algorithms for EEG signals includes detecting changes and irregularities (Epilepsy, Sleep and Emotions) in the EEG data. For example, *Orhan et al* [28] classified EEG signals using K-Means clustering and a multilayer Neural Network Model (NNM) for Healthy and Epileptic EEG data segments. K-means algorithm was used to obtain probability distributions of the wavelet coefficients which were to be inputted in the NNM for training. *Manjusha et al* [29] employed Machine learning algorithms like KNN classifier and K-Means algorithm to decrease false alarm rate and classification inaccuracies during EEG signal analysis. *Geva et al* [30] utilised unsupervised Fuzzy clustering to identify bioelectric states in the cerebral cortex to forecast anomalies like unusual transitions- gradual or sharp in nature which were indicative warnings for future problems like, impending psychotic

states, loss of vigilance in drivers, effect of hypoxia in pilots and also cardiac issues. *Hese et al* [31] employed K-Means algorithm for sleep stage classification by investigating relative band energy, harmonic parameters, and parameters of Hjorth which were used to extract relevant EEG. *Valenzi et al* [32] used Unsupervised methods like Vector Quantization, Fuzzy C-Means clustering, K-Means and K-Medians clustering to analyse EEG signals to classify emotional states. Similar work was done by *Murugappan et al* [33] classifying emotion using Fuzzy C-Means and Fuzzy K-Means algorithm which extracted features (to identify emotions) using methods based on multi-resolution analysis of wavelet functions. Clustering algorithms have therefore been widely used for classification and analysis of EEG data.

K-means

K-means is an exclusive partitioning clustering algorithm which operates in an unsupervised manner. The algorithm separates data in k-clusters of equal variances with an objective to minimize the within-cluster-sum-of-squares. It is a centroid based method, where it assigns each datapoint to a cluster whose centroid is the nearest. The algorithms are 3 basic steps: 1) An initial set of cluster centers (unless given an initial value) based on the specified number of K-clusters (input the number of K clusters). 2) With each iteration, every new datapoint is assigned to a cluster based on a distance metric (most common is Euclidean distance metric) and the cluster centers are recalculated. 3) Step 2 is repeated till the difference between the old and new centroids is lower than the specified threshold/tolerance value.

The pseudo code for the same is as follows shown in **Figure 2** [27].

Principal Component Analysis (PCA)

PCA is a Latent variable model technique used for dimensionality reduction of datasets (high dimensional data) with highly inter-related variables while still preserving the dataset's variability [34]. In layman terms, Dimensionality-reduction aids in easier exploration and visualization of data without compromising the uniqueness of the data. This is achieved by transforming the dataset into a new set of variables or principal components which are uncorrelated and ordered and yet retaining the variability of the original variables. The principal components are orthogonal eigenvectors of a covariance matrix. Interpretation of Principal components gives an idea about the inter-dimensional relationship (variables) of the data. The first principal component (PC1) retains the maximum variability of the data and the variability goes down for consecutive principal components. Visual representation of PCs with scatter plots aids in understanding data trends. PCs are often used as dependant variables for ANOVA and regression analysis. PCA is highly sought after in areas needing multivariate data analysis like quantitative finance, neuroscience, image compression, stock analysis and healthcare data analytics.

Materials and Methods

The approach taken in the present work is mentioned in **Figure 3** and defined below:

1) Data pre-processing

EEG data comprised of 8 waves (Delta, Theta, Alpha, Beta, Low-Beta, Mid-Beta, High-Beta and Gamma) per subject (50 subjects in total). The dataset was scaled for standardisation per subject, to fit the range of 0-1 individually with respect to each wave. The dataset contained missing data for Alpha wave, which was a result of artifact removal. The columns with the missing values were dropped, including the other waves in the column. The standardised pre-processed dataset per subject was collated for analysis.

2) Validation of Clustering Method (Single wave - Alpha)

Applying K-means algorithm Clustering Method for only the alpha waves is a trivial way of replicating the Supervised Learning Method of the previous study [17]. The idea was to check for the results with this approach and compare the same with the results from previous study and identify their convergence to verify the Clustering Methodology. In doing so, out of the total 50 subjects' data randomly selected 40

Input: S (dataset), K(number of cluster)
Initialize K clusters
while termination condition is not satisfied do
Find nearest centroid and assign datapoint *a* to the corresponding cluster
End for
Update cluster centroids using mean of the new clusters
Until stop iteration till criteria is satisfied
Output: Clusters/clustering result

Fig. 2 Figure showing the pseudo code for K-Means clustering algorithm [27].

Fig. 3 Figure showing the methodology implemented to analyse Olfactory stimulated EEG data using Unsupervised Machine Learning Algorithm.

subjects' data was taken as the training set and the rest 10 subjects' data form the validation set. We were able to divide the validation data into three clusters: 1) Relaxed set (AROMA, Lavender, ECO and ALO), 2) REST set (No action) and 3) ACT set (Timed Stress test). Euclidean distance metric was employed for K-means. Results from previous study were used for comparison.

3) Extension of the Clustering Method (Multi-wave Criterion)

K-means clustering was repeated for the above randomly chosen datasets – 40 training subjects and 10 validation subjects for waves of different frequencies. Euclidean distance metric was employed for K-means using the sci-kit learn library [27] with default parameters. Prediction accuracy is assessed as described above for the three clusters.

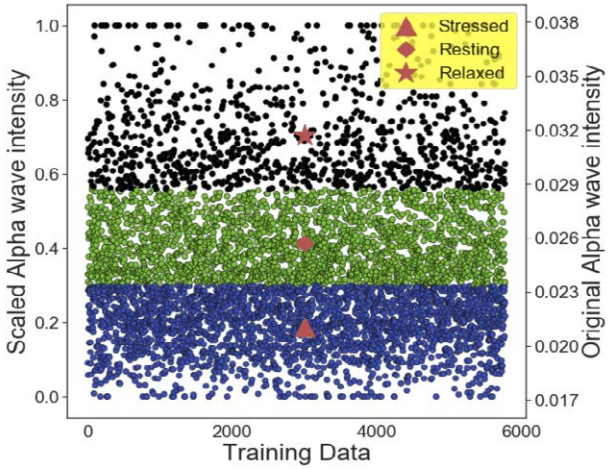

Fig. 4 Figure showing K-means clustering scatterplot for Alpha-waves with 3 distinct clusters (Stressed, Resting and Relaxed)

4) Principal Component Analysis (Multi-wave Criterion)

PCA was performed on the dataset to identify the combination of major frequency bands explaining the variance in the entire dataset. PCA was implemented using sci-kit learn library with default parameters [17]. PCA analysis was employed on EEG dataset to get the Eigenvalues, Cumulative variances, and Eigenvectors. The principal components sought in this investigation were a function of waves of different frequency bands. Contributions of each principal component towards the data (percentage variance) were obtained. Loading plots were used to depict this variance. Correlation of ACT, REST and Olfactory stimuli to Principal Components was obtained.

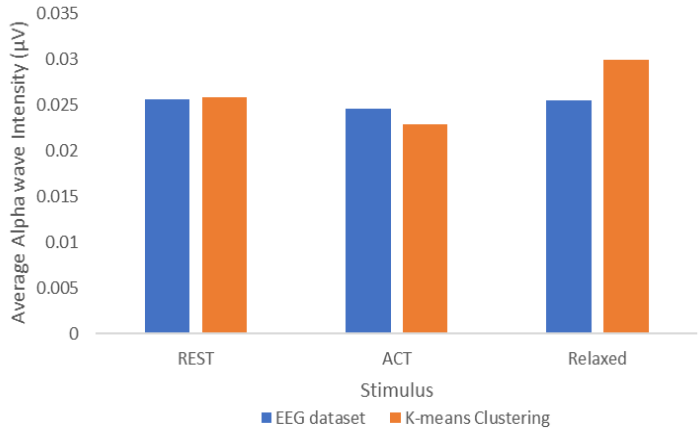

Fig. 5 Figure showing average Alpha Wave Mean Intensity (μV) for the 3 Clusters (K-Means Clustering) and, the corresponding groups from previous study (EEG dataset)

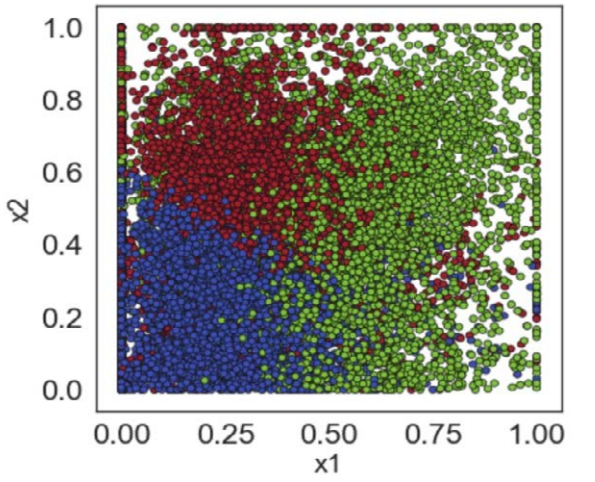

Fig. 6 Figure showing K-means Clustering with Multi-Waves Input(-considering all 8 waves)

5) Relaxation Potential Metric Proposition

Based on the above identification of principal components, a weighted average of the major principal components was obtained, and a new metric was proposed for the relaxation potential. The new metric was evaluated in terms of sensitivity and discrimination potential for different fragrances.

Results

Clustering Method Validation

K-Means Clustering algorithm for only the Alpha waves presented three distinct clusters as shown in **Figure 4**.

Three distinct clusters visible are characterized as: 1) Cluster A: $\alpha < 0.024$ μV 2) Cluster B: 0.024 μV $< \alpha < 0.029$ μV and 3) Cluster C: $\alpha > 0.029$ μV. Based on the learning from literature as described in the introduction, the lower the alpha waves more stressed the mental state. Hence the three

clusters are defined as mentioned in the **Figure 4**. The clusters obtained were then compared with the results from the previous work by *Kaushik et al* [17]. The Alpha waves intensity adapted from the previous study is compared with the centroid values of the three clusters as classified earlier in the present work. Same is represented in **Figure 5**.

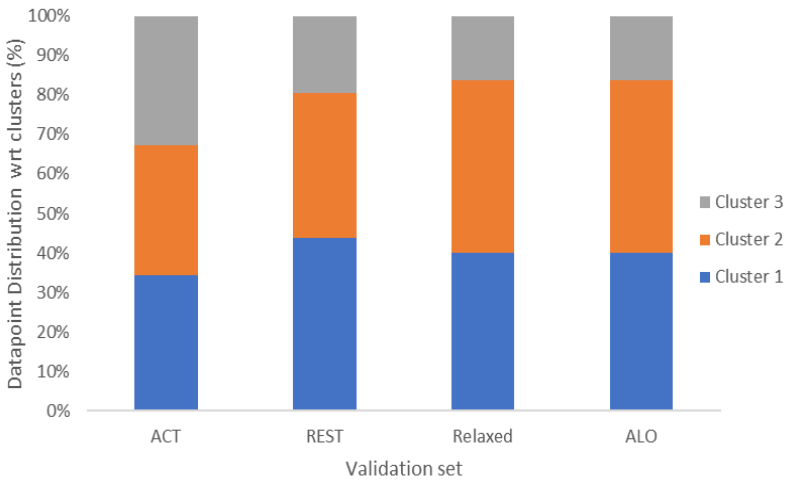

Fig. 7 Figure showing datapoint distribution (%) for K-Means clustering (all 8 brain waves). The histogram shows data overlap classified under ACT, REST, Relaxed and ALO stimuli.

Clustering Method Extension

K-means clustering done considering the waves from all the frequency bands. **Figure 6** shows the cluster representation of the data; the overlap amongst the clusters is evident from the figure.

Using the clustering information, the validation dataset was classified and mapped into the three clusters. **Figure 7** shows the overlap amongst the different clusters for the datapoints referring to one of the three states and ALO stimulus. The distribution of datapoints from each validation set towards the 3 clusters is shown here.

Principal Component Analysis (Multi-Wave Criterion)

PCA performed on the dataset segregated the top 4 Principal Components (PCs) explaining approximately 80% of the variability as shown in **Figure 8**.

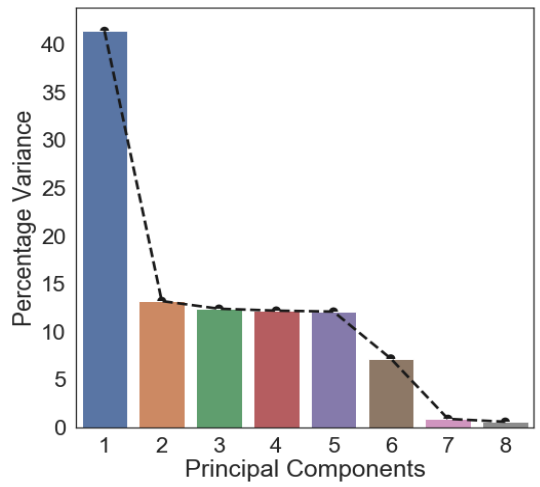

Fig. 8 Figure showing the modified Scree Plot defining the proportion of data variability, explained by the corresponding Principal Components

Loading plots which map the importance of different variables in terms of the effect on different principal components are graphed in **Figure 9**.

The coefficients for each variable for the two corresponding PCs are mapped on a 2-D plot. **Table 2** lists the coefficients of each variable for different principal components and conditional formatting is done for better visualization of the importance of each variable in different PCs.

Core data for PCs against each of the Olfactory Stimuli is averaged and captured in the **Table 3**.

As it can be seen from the formatting visualization, the interplay of the PCs is at display to describe a specific stimulus. This score data was then looked along with the Loading Data and specific insights were determined as discussed in the next section.

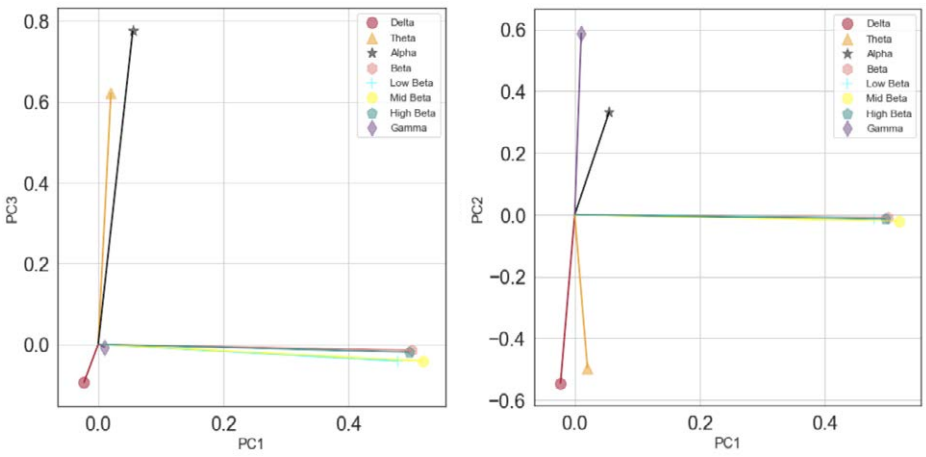

Fig. 9 Figure showing Loading Plots for Principal Components (PC) displaying the key variables for PC1, PC2 and PC3

Discussion

Alpha waves intensity in EEG measurement is representative of the degree of relaxation for a given stimulus. The same has been used as the relaxation metric in the previous study assessing the relaxation potential for four different fragrances. As understood from literature search, the relaxation behaviour of olfactive stimuli was governed by changes in waves from different frequency bands. We set out to apply unsupervised machine learning methods to decode the underlying combination of different known frequency bands to describe the change in brain waves on exposure to olfactive stimuli.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8
Delta	-0.023	-0.546	-0.094	0.801	0.228	-0.011	0.0	0.001
Theta	0.02	-0.495	0.621	-0.093	-0.599	0.005	-0.009	-0.005
Alpha	0.055	0.333	0.775	0.177	0.502	-0.036	0.002	0.001
Beta	0.501	-0.009	-0.014	0.014	-0.001	0.474	0.723	0.043
Low-Beta	0.479	-0.011	-0.041	-0.001	-0.027	-0.62	0.036	0.619
Mid-Beta	0.519	-0.021	-0.041	-0.007	-0.003	-0.367	-0.075	-0.767
High-Beta	0.496	-0.013	-0.019	0.004	0.021	0.506	-0.686	0.162
Gamma	0.01	0.587	-0.007	0.565	-0.579	0.008	-0.016	-0.014

Table 2 Table showing the coefficients of variable for each of the PCs (Principal Components)

As described earlier, the validation of Clustering K-Means Algorithm was done by taking a trivial case of clustering by using only the alpha waves data. Three distinct clusters were delineated, and the mean intensity was found to be similar to analysis listed in the previous study. Thus, the K-Means Clustering approach works in principle with single wave data analysis. Clustering followed the supervised rule – higher alpha value means a higher relaxed state – as described in the previous work [17]. However, when extended to multiple waves the K-Means Clustering Algorithm provides overlapping clusters and hence, reduced the prediction accuracy for the validation data set. The prediction accuracy was 34%, 19.4% and 43.04% for Stressed, Normal and Relaxed validation sets respectively, which was low. No distinct pattern/trend was observed in terms of the distribution of datapoints towards the clusters formed i.e., ACT validation set did not show any major proclivity towards a specific cluster. This suggests that the Clustering Method from Unsupervised Learning Techniques is not apt for the given dataset – owing to the less data points or sample size and inherent variations from person-to-person.

We then utilized Principal Component Analysis technique to reduce the dimensionality and identify key combinations of variables explaining maximum variance. We were able to identify 4 PCs explaining ~80% of variance. The principal waves were understood to be Alpha, Beta and Theta. This was in agreement with studies done by *Jensen et al* [35,36] and *Kilavik et al* [37] , where alpha, beta and theta wave changes were observed with activities dealing with information processing (processing the fragrance), memory tasks (stress test and mental correlational analysis of fragrance administered) and working memory load (stress test). By considering only the principal waves, we sought to reduce the wave noise. According to *Kilavik et al* [37], there was an increase in Beta power after static hold postures and post movement. Both these actions were included in the timed stress test, before the Normal/Rest activity. Therefore, the prediction results for Beta were also justified. The basis for this reasoning was from studies pertaining with visual and auditory stimulus. The current data was a result of visual stimulus from stress test and olfactory stimulus from fragrances which, has not been characterised yet in any study.

	PC 1	PC 2	PC 3	PC 4
REST	4.88	1.71	0.868	0.77
ACT	-2.12	-3.44	-2.87	-2.74
ALO	-1.55	-0.055	-1.03	-0.115
ECO	-0.717	3.21	1.04	2
LAVENDER	2.48	1.91	2.17	1.3
AROMA	2.21	2.06	4.45	1.12

Table 3 Table showing principal component score data for comparison of different experimental stimuli conditions

Table 4 details the PCs in terms of Brain wave intensity and help draw key insights listed below:

Principal Component	Cumulative contribution to Variability (%)	Characterization of PCs in terms of Brain waves intensity
PC 1	41.4	High (Beta + Low Beta + Mid Beta + High Beta)
PC 2	54.6	Low (Delta + Low Theta) + Mid (Alpha) + High (Gamma)
PC 3	67	High (Theta + Alpha)
PC 4	79.2	High (Delta + Gamma) + Mid (Alpha)
PC 5	91.3	High (Alpha) + Mid (Delta) + Low (Theta + Gamma)
PC 6	98.5	High (Beta + High Beta) + Low (Low Beta + Mid Beta)
PC 7	99.4	High (Beta) + Low (High Beta)
PC 8	100	High (Low Beta) + Mid (High Beta) + Low (Mid Beta)

Table 4 Table showing Principal Components' Characteristics in terms of Brain wave intensity (High and/or Low)

- PC3 had the highest Alpha wave intensity contribution and strongest correlation to AROMA stimulus. This meant AROMA fragrance did induce higher state of relaxation, followed by Lavender and ECO

- PC3 was a combination of high Theta and Alpha wave intensity. High Theta waves intensities have not yet been systematically correlated with any particular mental state. Although the textbook indications for low Theta wave intensities are Drowsiness and REM Sleep. According to *Schacter et al* [38], high Theta wave intensity was observed for activities concerned with both meditation and concentration. Therefore, it was difficult to characterise High Theta wave intensity value.

- PC1 had a High Beta Wave Intensity (includes Low Beta, Mid Beta & High Beta) which strongly correlated with Rest activity. This was justified as (Low-Beta) and (High Beta) wave intensities were aroused during mental states of Fast Idle, musing, complex thoughts and integrating new experience. High Beta wave intensity was known to be induced post-movement/action [37].

- PC2 and PC4 both had high Gamma wave intensity. High Gamma wave intensity was an indication of meditative mental states. A steady increase in Gamma wave intensity was detected during the course of meditation practices **(Table 1)**. PC2 and PC4 had the highest correlation to ECO.

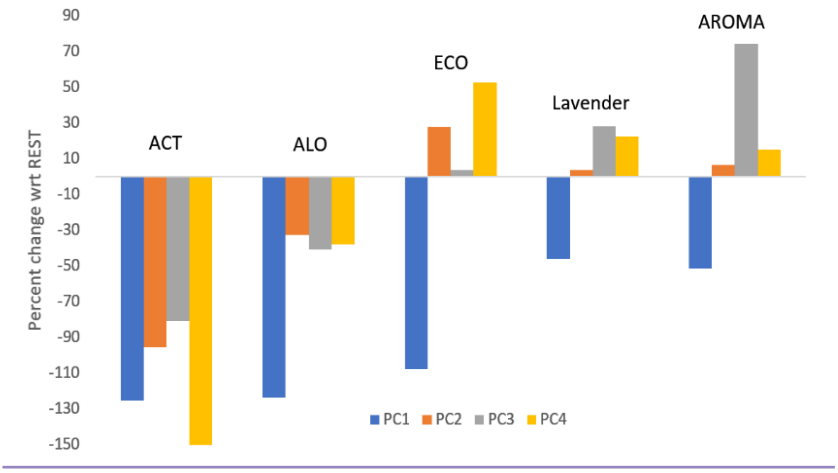

Fig.10 Figure showing the percent change from Resting State for different Olfactive Exposures (ALO, ECO, Lavender and AROMA) and Stress test (ACT) for PC1, PC2, PC3 and PC3 (~80% data variability)

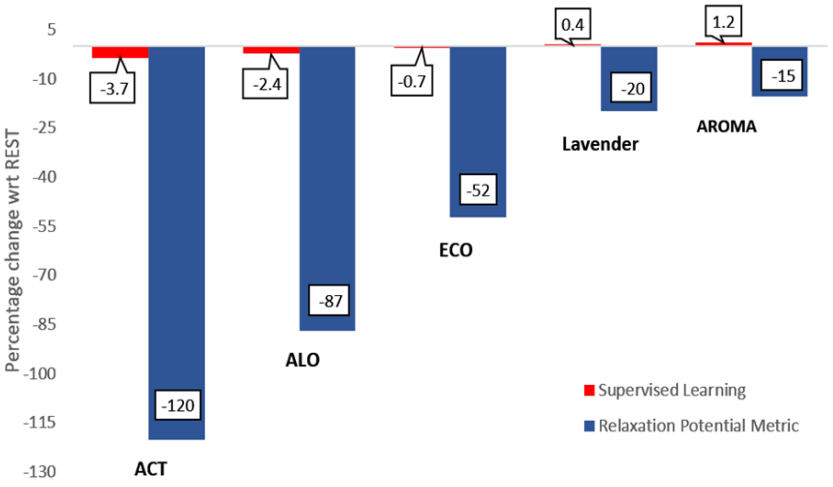

Fig.11 Figure showing the percent change of Olfactive exposure and Stress test (ACT) for weighted average principal components and supervised learning wrt Resting state as the Baseline.

Relaxation Potential Metric

Principal Component changes from the rest state with respect to various olfactive stimuli is listed in **Figure 10**. Percentage change for PC3 was very well spread out: AROMA (+77%), Lavender (+28%), ECO (+3.6%) and ACT (-95%). Thus, it helps discriminate the olfactive stimuli much better. PC3, as explained above, is a positively correlated with Theta and Alpha Waves. Thus, it can be used as a discriminant metric in place of only the Alpha wave as specified in the previous work. However, it does not explain the sudden improvement of no fragrance – placebo (ALO) over the ACT stimulus as well as the high discrimination between Lavender and synergistic blend of essential oils (AROMA).

Another way to look at a relaxation metric is to obtain a weighted average Principal Component with weights equivalent to the corresponding Scree Plot Variances explained by specific PC. The same if exhibited in **Figure 11** above. The weighted average PC provides a broader range to discriminate the smaller changes caused by somewhat similar stimuli.

Conclusion

This study presents a new approach in analysing the EEG data for Olfactory Stimuli in terms of their potential to induce relaxation benefit in humans. We built upon the data from a previous study and explored Unsupervised Machine Learning techniques to identify the methodology best suited given the constraint of data points and inherent variances from subject-to-subject. We used Principal Component Analysis to identify the best combination of different frequency bands (brain wave classes) explaining the maximum variance in the

data due to olfactive stimuli. We concluded that it is significant to consider the contribution of all brain waves to define the variance observed for olfactory stimulus than only considering alpha waves. We proposed a new relaxation potential metric as the weighted average of principal components and, used it to compare the degree of relaxation benefit from different olfactive stimuli from the previous study. The new relaxation metric was able to discriminate amongst the olfactive stimuli better than just the alpha waves, as done in the previous study. The approach defined in this work can be used as a tool to analyse & discriminate the variance induced in brain waves on exposure to one or more sensory stimuli. This can be a good tool for product development researchers while designing their products for superior, consumer-perceivable sensory benefits.

References:

[1] *Meerwein, Gerhard; Rodeck, Bettina; Mahnke, Frank H.* (2007): Color. Communication in architectural space / Meerwein, Rodeck, Mahnke. Basel: Birkhäuser.

[2] *Price, Shirley; Price, Len* (2012): Aromatherapy for health professionals. 4th ed. Edinburgh, New York: Churchill Livingstone/Elsevier.

[3] Merriam-Webster. (n.d.). Olfaction. In Merriam-Webster.com dictionary., from https://www.merriam-webster.com/dictionary/olfaction

[4] *March, Claire A. de; Ryu, SangEun; Sicard, Gilles; Moon, Cheil; Golebiowski, Jérôme* (2015): Structure-odour relationships reviewed in the postgenomic era. In Flavour Fragr. J. 30 (5), pp. 342–361. DOI: 10.1002/ffj.3249.

[5] Schacter, Daniel L.; Gilbert, Daniel Todd; Wegner, Daniel M. (2011): Psychology. 2nd ed. New York, NY: Worth Publishers.

[6] The Mechanism of Olfaction (2014). In Charles S. Sell (Ed.): Chemistry and the Sense of Smell. Hoboken, NJ, USA: John Wiley & Sons, Inc, pp. 32–187.

[7] *Hongratanaworakit, Tapanee* (2011): Aroma-therapeutic Effects of Massage Blended Essential Oils on Humans. In Natural Product Communications 6 (8), 1934578X1100600. DOI: 10.1177/1934578X1100600838.

[8] *Sánchez-Vidaña, Dalinda Isabel; Ngai, Shirley Pui-Ching; He, Wanjia; Chow, Jason Ka-Wing; Lau, Benson Wui-Man; Tsang, Hector Wing-Hong* (2017): The Effectiveness of Aromatherapy for Depressive Symptoms: A Systematic Review. In Evidence-Based Complementary and Alternative Medicine.

[9] *Hongratanaworakit, Tapanee* (2004): Physiological effects in aromatherapy. In Songklanakarin Journal of Science & Technology 26 (1), pp. 117–125.

[10] *St. Louis, Erik K.; Frey, Lauren C.; Britton, Jeffrey W.* (Eds.) (2016): Electroencephalography (EEG). An introductory text and atlas of normal and abnormal findings in adults, children, and infants. Chicago, IL: American Epilepsy Society.

[11] *Empson, Jacob* (1986): Human Brainwaves. The Psychological Significance of the Electroencephalogram: Springer,page 1-20.

[12] *Subha, D. Puthankattil; Joseph, Paul K.; Acharya U, Rajendra; Lim, Choo Min* (2010): EEG Signal Analysis: A Survey. In J Med Syst 34 (2), pp. 195–212. DOI: 10.1007/s10916-008-9231-z.

[13] *Lutz, Antoine; Greischar, Lawrence L.; Rawlings, Nancy B.; Ricard, Matthieu; Davidson, Richard J.* (2004): Long-term meditators self-induce high-amplitude gamma synchrony during mental practice. In Proceedings of the National Academy of Sciences of the United States of America 101 (46), pp. 16369–16373. DOI: 10.1073/pnas.0407401101.

[14] *Belkofer, Christopher M.; van Hecke, Amy Vaughan; Konopka, Lukasz M.* (2014): Effects of Drawing on Alpha Activity: A Quantitative EEG Study With Implications for Art Therapy. In Art Therapy 31 (2), pp. 61–68. DOI: 10.1080/07421656.2014.903821.

[15] *Phneah, Swee Wu; Nisar, Humaira* (2017): EEG-based alpha neurofeedback training for mood enhancement. In Australas Phys Eng Sci Med 40 (2), pp. 325–336. DOI: 10.1007/s13246-017-0538-2.

[16] *Sowndhararajan, Kandhasamy; Kim, Songmun* (2016): Influence of Fragrances on Human Psychophysiological Activity: With Special Reference to Human Electroencephalographic Response. In Sci. Pharm. 84 (4), pp. 724–751. DOI: 10.3390/scipharm84040724.

[17] *Kaushik, Vaibhav; Tungekar, Imad; Ingle, Rahul; Mhatre, Shilpa; Awale, Raval; Mhaskar, Sudhakar; Kulkarni, Amol* (In Press). Effect of Coconut Oil Aroma on EEG Activity and Relaxation State in Healthy Human Beings: A Clinical Study. SOFW-Journal ; Electrical Engineering, Veermata Jijabai Technological Institute; Marico R&D Center, Marico Limited.

[18] *Field, Tiffany; Diego, Miguel; Hernandez-Reif, Maria; Cisneros, Wendy; Feijo, Larissa; Vera, Yanexy et al.* (2005): Lavender Fragrance Cleansing Gel Effects on Relaxation. In International Journal of Neuroscience 115 (2), pp. 207–222. DOI: 10.1080/00207450590519175.

[19] *Diego, Miguel A.; Jones, Nancy Aaron; Field, Tiffany; Hernandez-Reif, Maria; Schanberg, Saul; Kuhn, Cynthia et al.* (1998): Aromatherapy Positively Affects Mood, Eeg Patterns of Alertness and Math Computations. In International Journal of Neuroscience 96 (3-4), pp. 217–224. DOI: 10.3109/00207459808986469.

[20] *Lorig; S, Tyler; Schwartz, Gary E.* (1988): Brain and odor: I. Alteration of human EEG by odor administration. In Psychobiology 16 (3), pp. 281–284. DOI: 10.3758/BF03327318.

[21] *Lorig, Tyler S.; Schwartz, Gary E.; Herman, Kate B.; Lane, Richard D.* (1988): Brain and odor: IL EEG activity during nose and mouth breathing. In Psychobiology 16 (3), pp. 285–287. DOI: 10.3758/BF03327319.

[22] *Klemm, W. R.; Lutes, S. D.; Hendrix, D. V.; Warrenburg, Stephen* (1992): Topographical EEG maps of human responses to odors. In Chem Senses 17 (3), pp. 347–361. DOI: 10.1093/chemse/17.3.347.

[23] *Maimon, Oded.; Rokach, Lior. (Eds.)* (2005): Data Mining and Knowledge Discovery Handbook. Boston, MA: Springer US.

[24] *Nielsen, Frank* (2016): Introduction to HPC with MPI for Data Science. 1st ed. 2016. Cham: Springer (Undergraduate Topics in Computer Science).

[25] *Chen, Chien-Yu; Hwang, Shien-Ching; Oyang, Yen-Jen* (2002): An incremental hierarchical data clustering algorithm based on gravity theory. With assistance of Pacific-Asia Conference on Knowledge Discovery and Data Mining. Berlin, Heidelberg: Springer.

[26] *Patra, Bidyut Kr.; Ville, Ollikainen; Launonen, Raimo; Nandi, Sukumar; Babu, Korra Sathya* (2013): Distance based Incremental Clustering for Mining Clusters of Arbitrary Shapes. In Pradipta Maji (Ed.): Pattern recognition and machine intelligence. 5th International Conference, PReMI 2013, Kolkata, India, December 10-14, 2013, proceedings / Pradipta Maji [and four others] (eds.), vol. 8251. Heidelberg: Springer (LNCS sublibrary. SL 6, Image processing, computer vision, pattern recognition and graphics, 8251), pp. 229–236.

[27] Scikit-learn: Machine Learning in Python, Pedregosa et al., JMLR 12, pp. 2825-2830, 2011.

[28] *Orhan, Umut; Hekim, Mahmut; Ozer, Mahmut* (2011): EEG signals classification using the K-means clustering and a multilayer perceptron neural network model. In Expert Systems with Applications 38 (10), pp. 13475–13481. DOI: 10.1016/j.eswa.2011.04.149.

[29] *Manjusha, M.; Harikumar, R.* (2016 - 2016): Performance analysis of KNN classifier and K-means clustering for robust classification of epilepsy from EEG signals. In : 2016 International Conference on Wireless Communications, Signal Processing and Networking (WiSPNET). Chennai, 23-03-2016 - 25-03-2016: IEEE, pp. 2412–2416.

[30] *Geva, A. B.; Kerem, D. H.* (1998): Forecasting generalized epileptic seizures from the EEG signal by wavelet analysis and dynamic unsupervised fuzzy clustering. In IEEE Trans. Biomed. Eng. 45 (10), pp. 1205–1216. DOI: 10.1109/10.720198.

[31] *van Hese, Peter; Philips, Wilfried; Koninck, J. de; van de Walle, Rik; Lemahieu, Ignace* (2001): Automatic detection of sleep stages using the EEG. In 23rd annual international conference of the IEEE engineering in medicine and biology society 2, pp. 1944–1947.

[32] *Valenzi, Stefano; Islam, Tanvir; Jurica, Peter; Cichocki, Andrzej* (2014): Individual Classification of Emotions Using EEG. In Journal of Biomedical Science and Engine. 7 (8), pp. 604–620. Available online at http://www.scirp.org/journal/PaperInformation.aspx?PaperID=4736.

[33] *Murugappan, M.; Rizon, M.; Nagarajan, R.; Yaacob, S.* (2010): Inferring of human emotional states using multichannel EEG. In European Journal of Scientific Research. 48 (2), 281-199.

[34] *Bartholomew, David J.* (2011): Analysis of multivariate social science data. 2nd ed. Boca Raton, Florida: CRC Press (Chapman & Hall/CRC Statistics in the Social and Behavioral Sciences).

[35] *Jensen, Ole; Tesche, Claudia D.* (2002): Frontal theta activity in humans increases with memory load in a working memory task. In European Journal of Neuroscience 15 (8), pp. 1395–1399. DOI: 10.1046/j.1460-9568.2002.01975.x.

[36] *Jensen, O.* (2002): Oscillations in the Alpha Band (9-12 Hz) Increase with Memory Load during Retention in a Short-term Memory Task. In Cerebral Cortex 12 (8), pp. 877–882. DOI: 10.1093/cercor/12.8.877.

[37] *Kilavik, Bjørg Elisabeth; Zaepffel, Manuel; Brovelli, Andrea; MacKay, William A.; Riehle, Alexa* (2013): The ups and downs of beta oscillations in sensorimotor cortex. In Experimental neurology 245, pp. 15–26. DOI: 10.1016/j.expneurol.2012.09.014.

[38] *Schacter, Daniel L.* (1977): EEG theta waves and psychological phenomena: A review and analysis. In Biological Psychology 5 (1), pp. 47–82. DOI: 10.1016/0301-0511(77)90028-X.

authors

Chinmay Mhaskar [1], Vaibhav Kaushik [2], Archana Arjula [3]

[1] Department of Chemical and Biochemical Engineering, Rutgers University New Brunswick | New Jersey | USA

[2] Marico R&D Center | 23-C, Mahal Industrial Estate | Mahakaali Caves Road Andheri (E) – Mumbai 400 093 | INDIA

[3] Affiliated to Department of Electrical and Computer Engineering, Rutgers University - New Brunswick | New Jersey | USA

Corresponding author: Chinmay Mhaskar
Rutgers University - New Brunswick | New Jersey | United States
csmhaskar@gmail.com

Appendix

Clustering methods are frequently used as unsupervised learning algorithms. The method of clustering can either be Hierarchical or partitional in nature. When successive clusters are determined based on previously established clusters then the algorithm is hierarchical in nature and when, all the clusters are determined in one go the algorithm is partitional in nature [1]. K-Means, Affinity propagation, Hierarchical, Agglomerative, DBSCAN, OPTICS and Gaussian mixtures are most frequently used [2].

Anomaly detection methods identify deviations/noise/outliers/novelties/ exception or all, in data. Real-life applications for this method would be to detect errors in structured and well-defined autonomous and non-autonomous activities like fraud detection, intrusion detection, network performance, structural defect detection, time-series monitoring and medical condition monitoring. Popular techniques for the same are density-based techniques, ensemble-based techniques, cluster analysis techniques, Bayesian networks and Hidden Markov models (HMMs). [3-9]

Neural networks are used for estimation problems which concern with clustering, statistical distributions, compression and filtering the input. USL using Neural networks employs autoencoders, Deep belief networks, Hebbian learning, Generative adversarial networks (GANs) and self-organizing maps (Kohonen networks). Autoencoders use dimensionality reduction to ignore the noise in the data and form a representation on the input in an unsupervised way. Deep belief networks are a type of deep neural network composed of multiple layers of hidden units where the layers are interconnected. The training for such networks happens unsupervised so that the inputs can be probabilistically reconstructed [10]. Hebbian learning is based on a neuroscientific theory. The basic principle of the theory attempts to describe working of brain's neurons during the learning process, where repetitive and persistent inputs to postsynaptic cell through a presynaptic cell decides the synaptic efficacy [11]. GANs when inputted with a training set learn to generate new data representing similar statistics. It trains in an indirect way using a discriminator. Kohonen networks use USL during training to represent the input as low-dimensional discretized input space. Essentially it is another method for dimensionality reduction.

Latent variable model is a statistical technique that relates observable variable to latent variables. These models are used for dimensionality reduction and clustering techniques. The models can be classified as categorical or continuous for both latent and manifest variables [12].

EM algorithm is a maximum-likelihood method estimation using latent variables. The algorithm predicts machine learning model parameters. The algorithm iteratively finds maximum likelihood estimates of the required parameters in statistical models. There is an expectation (E) step and maximization (M) step. The E step estimates the missing/latent variables in the dataset. The estimate is for the process latent variable for each data point. The M step optimizes the model parameter of to maximise the expected values. The algorithm iterates between the two steps to maximize the expected values.

Blind signal separation is a technique to separate a set of source signals from multivariate/mixed signals with much information about the source. The keyword blind means that the separation is done on the basis of the given data without knowing the nature of the data structure. PCA and ICA are the most common techniques for such data separations. PCA (Principal component

Analysis) is commonly used for dimensionality reduction for lower dimensional data while preserving the variation in the data. The algorithm is very fundamental in machine learning and has applications in areas ranging from quantitative finance to computer vision. ICA (Independent Component Analysis) separates a multivariate signal to subcomponents. The components are additive and non-Gaussian in nature. ICA looks to maximize the independence (statistical) of estimated components. The independence is defined based on maximizing the non-Gaussian nature of the components and minimizing the mutual information (measure of mutual dependence between variables). ICA applications include stock price rediction, artifact removal from EEG data, face recognition and neuron optical imaging [13][14][15].

Clustering methods are the most common type of unsupervised learning algorithms which group the data based on similarity metrics like (Euclidean distance ,Manhattan distance metric or other distance metric). Anomaly detection methods identify deviations/noise/outliers/novelties/ exception or all, in data for example, fraud detection, intrusion detection, network performance, structural defect detection, time-series monitoring and medical condition monitoring[16]. Neural networks are used for estimation problems which concern with clustering, statistical distributions, compression and filtering the input. USL using Neural networks employs autoencoders, Deep belief networks, Hebbian learning, Generative adversarial networks (GANs) and self-organizing maps (Kohonen networks)[10][11]. Latent variable model is a statistical technique that relates observable variable to latent variables. These models are used for both dimensionality reduction and clustering techniques.EM algorithm, PCA (Principal component Analysis) and ICA(Independent Component Analysis) can be classified under this category.[17][13][18].

References

[1] *Madhulatha, T. Soni*: An Overview on Clustering Methods. In IOSR Journal of Engineering 2(4) pp: 719-725. Available online at https://arxiv.org/pdf/1205.1117.

[2] Scikit-learn: Machine Learning in Python, Pedregosa et al., JMLR 12, pp. 2825-2830, 2011.

[3] *Knorr, Edwin M.; Ng, Raymond T.; Tucakov, Vladimir* (2000): Distance-based outliers: algorithms and applications. In The VLDB Journal The International Journal on Very Large Data Bases 8 (3-4), pp. 237–253. DOI: 10.1007/s007780050006.

[4] *Ramaswamy, Sridhar; Rastogi, Rajeev; Shim, Kyuseok* (2000): Efficient algorithms for mining outliers from large data sets. In Maggie Dunham, Jeffrey F. Naughton, Weidong Chen, Nick Koudas (Eds.): Proceedings of the 2000 ACM SIGMOD international conference on Management of data - SIGMOD '00. the 2000 ACM SIGMOD international conference. Dallas, Texas, United States, 5/15/2000 - 5/18/2000. New York, New York, USA: ACM Press, pp. 427–438.

[5] *Elomaa, Tapio; Mannila, Heikki; Toivonen, Hannu* (2002): Principles of data mining and knowledge discovery. 6th European conference, PKDD 2002, Helsinki, Finland, August 19-23, 2002 : proceedings / Tapio Elomaa, Heikki Mannila, Hannu Toivonen (eds.). Berlin, London: Springer (Lecture notes in computer science, Lecture notes in artificial intelligence 0302-9743, 2431).

[6] *Dunham, Maggie* (2000): Proceedings of the 2000 ACM SIGMOD International Conference on Management of Data. 2000, Dallas, Texas, United States, May 15- 8, 2000. [New York]: Association for Computing Machinery. Available online at http://dl.acm.org/citation.cfm?id=342009.

[7] *Kambayashi, Yahiko; Winiwarter, Werner; Arikawa, Masatoshi (Eds.)* (2002): Data warehousing and knowledge discovery. 4th international conference, DaWaK 2002, Aix-en-Provence, France, September 4-6, 2002 : proceedings / *Yahiko Kambayashi, Werner Winiwarter, Masatoshi Arikawa (eds.)*. Berlin, London: Springer (Lecture notes in computer science, 0302-9743, 2454).

[8] *Campello, Ricardo J. G. B.; Moulavi, Davoud; Zimek, Arthur; Sander, Jörg* (2015): Hierarchical Density Estimates for Data Clustering, Visualization, and Outlier Detection. In ACM Trans. Knowl. Discov. Data 10 (1), pp. 1–51. DOI: 10.1145/2733381.

[9] *Kitawaga, Hiroyuki* (2010): Database systems for advanced applications. 15th international conference, DASFAA 2010, Tsukuba, Japan, April 1-4, 2010, proceedings / Hiroyuki Kitagawa … [et al.]. Berlin: Springer (Lecture notes in computer science, 0302-9743, 5981-5982). Available online at http://www.springer.com/gb/ BLDSS.

[10] *Hinton, Geoffrey* (2009): Deep belief networks. In Scholarpedia 4 (5), p. 5947. DOI: 10.4249/scholarpedia.5947.

[11] *Morris, R.G.M* (1999): D.O. Hebb: The Organization of Behavior, Wiley: New York; 1949. In Brain Research Bulletin 50 (5-6), p. 437. DOI: 10.1016/S0361-9230(99)00182-3.

[12] Bartholomew, David J. (2011): Analysis of multivariate social science data. 2nd ed. Boca Raton, Florida: CRC Press (Chapman & Hall/CRC Statistics in the Social and Behavioral Sciences).

[13] *Clifford GD.* Blind Source Separation: Principal & Independent Component Analysis. Chapter 15 for HST-582J/6.555J/16.456J Biomedical Signal and Image Processing 2005-2018.

[14] *Stone, James V.* (2004): Independent component analysis. A tutorial introduction / James V. Stone. Cambridge, Mass., London: MIT Press.

[15] *Hyvärinen, Aapo* (2013): Independent component analysis: recent advances. In Philosophical transactions. Series A, Mathematical, physical, and engineering sciences 371 (1984), p. 20110534. DOI: 10.1098/rsta.2011.0534.

[16] *Bartholomew, David J.* (2011): Analysis of multivariate social science data. 2nd ed. Boca Raton, Florida: CRC Press (Chapman & Hall/CRC Statistics in the Social and Behavioral Sciences).

[17] *Hyvärinen, Aapo; Karhunen, Juha; Oja, Erkki* (2001): Independent Component Analysis. 1st ed. S.l.: Wiley-Interscience (Adaptive and learning systems for signal processing, communications, and control). Available online at http://site.ebrary.com/lib/alltitles/docDetail.action?docID=10272411.

"Our ambition? Excellence"

Prof. Wilhelm about the new proderm design

2 years ago, on the occasion of our 25th anniversary, I announced our vision for the future development of proderm under the heading 'Setting the course and company foundation 2.0'. In addition to significant investments, a route to digitalization and plans for process optimizations, it was also about flexibility and adaptability to new challenges. We now also want to clearly express this by changing our design.

Starting in September this year, we will present ourselves with a new look. It will display familiar aspects in a fresh, modern design while simultaneously conveying two core elements of our identity: continuity and change. What does this mean?

Simpler, faster, better – proderm

proDERM® Institut für Angewandte Dermatologische Forschung GmbH is becoming **proderm®**. The simplification of our company name is a result of our ambition to be **simpler**, **faster**, **better** – and because most of our customers and partners think of us and address us "only" as **proderm®**. Furthermore, the company name contains an important message: despite our expansion to different indication areas, our focus continues to be on dermatology.

Our performance standards remain unchanged! When my wife and I established **proderm®** in 1994, we defined a solid basis of principles that defined the working method of our institute.

Scientific integrity, professionalism, a sense of responsibility and a culture of continual improvement are in our company DNA. We continue to express this in our logo with the familiar pyramid. We live these values – and will do so in the future.

No compromises on quality and reliability

The pyramid – now lighter, fresher and a bit more abstract – also reflects the agility and diversity of our company. Moreover, it stands upright, symbolising our philosophy and vision in addition to our high standards for our own performance. **proderm®** provides high-quality services and is the first choice for contractors worldwide who accept no compromises on quality and reliability in their contract research on indications for dermatology, ophthalmology, tooth and mouth hygiene and women's health.

"Our ambition? Excellence"

Last but not least: our new slogan. **"Our ambition? Excellence"** will express our identity even more clearly in the future. We do not simply rest on our laurels. Instead, we optimise and invest, set new standards, actively participate in scientific discussions and "go the extra mile" for excellent service.

Now, after more than 25 years, **proderm®** will have a new design starting in September. We're looking forward to it – as we look forward to continued successfully supporting our clients with their exciting projects.

Yours faithfully,

Klaus-Peter Wilhelm
www.proderm.de

Klaus-Peter Wilhelm

DGP Spring Conference 2021

E. Diaz, A. Wilsch-Irrgang

DEUTSCHE GESELLSCHAFT DER PARFÜMEURE im SEPAWA® e.V.

Despite the ongoing corona pandemic, the **2021 DGP Spring Conference** could take place - though this time virtually. In a zoom conference perfectly organized by **SEPAWA® e.V.**, 50 participants had gathered on **March 19, 2021**, to attend a morning of topical lectures and to exchange ideas.

The conference started with an overview of the **DGP** activities of the past two years delivered by the **DGP President *Edison Diaz***.

Professor Andrea Büttner from the **Fraunhofer Institute for Process Engineering and Packaging** gave insights into the broad task of her institute titled "*Sensing and Making Sense of a Changing World*".

The goal is to develop sustainable products that serve people's desires, but do so without any long-term impact on the environment or health. This requires collaboration between a wide range of disciplines.
The interaction between humans and machines is to be expanded with the development of powerful sensor technology for olfactory impressions and other sensory perceptions. Research fields include, for example, chemocommunication, since body odors can provide information about diseases.
The technical recording of multimodal sensory impressions is also very important in many other areas, for example to detect product counterfeiting or to check the suitability of raw materials when recycling plastics. For perfumers, the call to make product fragrances recyclable at the design stage is likely to attract attention.

The second presentation dealt with a different aspect of human-machine collaboration. ***Ms. Claire Viola*, Symrise AG**, reported on *Philyra* under the title "*Breaking New Fragrance Ground with Artificial Intelligence.*" Together with **IBM Research**, the company developed a method in 2018 that uses artificial intelligence (AI) to support perfumers.

Even if a fragrance composition deviates only marginally from the specified amounts, it either creates a new fragrance or destroys the overall work. *"Philyra"* uses a data-driven approach and accesses a huge database of fragrance formulas, data on fragrance ranges, as well as historical data. This uses artificial intelligence to create a fragrance specifically for Brazilian men of the Millennial generation, for example, from the treasure chest of data. AI identifies existing fragrances and suggests additional components and formulas. The perfumer receives such suggestions, then thereby accentuates certain fragrance notes, optimizes the fragrance impression, and from this combined, new types of fine fragrances are created.
Philyra understands consumer demands and knows formulas as well as raw materials. This results in new fragrance combinations and accelerates the perfumers' creative process. They can now concentrate on refining the final products. *Ms. Viola* explained *Philyra* as the leading innovation in the fragrance industry.
During the networking break that followed, the participants met in two chat rooms where, amongst other things, there was further intensive discussion about *Philyra* with *Claire Viola*.

This was followed by the election of the new **DGP board**. Under the leadership of **SEPAWA® e.V. Chairman *Hans-Jürgen Scholz***, the DGP members confirmed ***Edison Diaz***, ***Anneliese Wilsch-Irrgang*** and ***Carolin Sturm*** in their previous functions. ***Lars Schlüter*** was newly elected for the budget department. ***Daniel Dillenséger,*** who was previously responsible for the department, left the board. He continues to support the DGP in the Advisory Board. *Edison Diaz* expressed his heartfelt thanks for his many years of knowledgeable and constructive work on the DGP Board and presented him with a certificate of honor.

The following lecture by ***Professor Thomas Hummel*, University of Dresden**, showed the latest scientific findings on smelling and olfactory disorders under the title *"Smell and Smell Dysfunction in the Corona Pandemic"*. An impressive tracking shot of the nose led into the effects of a Covid-19 infection on the ability to smell. About 60% of all Covid sufferers experience olfactory dysfunction, and in 10% this is actually the main effect of the infection. The virus causes inflammation of both the olfactory cells and the olfactory nerve.

Loss of sense of smell is well indicated as an early indication of covid infection. Most olfactory disorders are reversible, and recovery takes approximately 4 to 8 weeks. For faster recovery, administration of vitamin A as well as specific olfactory training are beneficial.

During healing, panosmia (false olfactory impressions) or phantosmia (one smells something without an odor being present) may initially occur - these are to be regarded as good signs!
In an excursion, *Professor Hummel* also presented the latest research approaches for the treatment of irreversible olfactory loss, e.g. transplantation of olfactory epithelial cells. Just as after the preceding presentations, a lively discussion ensued, in which one participant also shared her personal experience of the positive effects of olfactory training.

In conclusion, the focus then shifted from the human health to the health of the economy. *"Retail in Lockdown - How Corona is Changing the Industry"* was the title of the presentation by ***Mr. Elmar Keldenich*, Managing Director of the German Perfumery Association**.

In a virtual time journey from the beginning of 2019 to the end of 2020, *Mr. Keldenich* provided information about the shifts that Corona generated in the retail landscape. Perfumeries were particularly affected here with their locations primarily in city centers and the targeted product selection during the lockdowns. In the first lockdown in 2020, drugstores benefited particularly with sales of hygiene products. Perfumeries made less than 50% of the previous year's sales in April 2020. In this phase, suburbs gained, whereas city centers and large shopping malls lost out.

In the fall of 2020, shopping behavior continued to change, from shopping to utility shopping. Consumers preferred one-stop shopping, drugstores suffered losses, and supermarkets offering a wide range of everyday items from food to hygiene and cosmetics recorded gains in sales.
Perfumeries were closed on 100 days in 2020, and recorded a 16% drop in sales compared with the previous year. Online sales increased by 20%, but are still low in absolute terms.
In the future, it is expected that more new, creative fragrances will be established in the personal fragrance sector that do not just cater for mass tastes, and that niche perfumery will continue to grow.

After four and a half informative, interactive and stimulating hours, *Edison Diaz* closed the virtual **DGP Spring Conference 2021** with a big thank you to all presenters, to the participants and to the **SEPAWA® e.V.** organization in person of ***Madeline Dettenrieder***.

Contact

Dr. Edison Diaz
Symrise AG
Mühlfeldstr. 1 37603 | Holzminden | Germany

CC-Eye perfector

Concealer | CC-FR-21-BC-50872-05

The perfect solution to take care of your eye contour in one unique ritual thanks to the CC-eye perfector concealer, offering both color correction with pigments and bioactive and a fresh relooking with instant results.

Phase	Ingredients	INCI	% by weight	Function
A	Dehymuls® PGPH	Polyglyceryl-2 Dipolyhydroxystearate	5.00	Emulsifier (W/O)
	Lameform® TGI	Polyglyceryl-3 Diisostearate	2.00	Emulsifier (W/O)
	Cutina® PES	Pentaerythrityl Distearate	1.50	Structurant
	Cosmedia® Gel CC	Dicaprylyl Carbonate, Stearalkonium, Hectorite, Propylene Carbonate	7.00	Rheology modifier
	Cetiol® Sensoft	Propylheptyl Caprylate	7.00	Emollient
	DOWSIL 9041 Silicone Elastomer Blend (*Dow Corning*)	Dimethicone, Dimethicone Crosspolymer	2.00	Skin feel modifier
B	Water, demin.	Aqua	56.94	
	1,3-Butanediol	Butylene Glycol	2.00	Humectant
	Magnesium Sulfate	Magnesium Sulfate	1.00	Stabilizer
	Preservative		q.s.	Preservative
C	Cetiol® A	Hexyl Laurate	4.00	Emollient
	Cetiol® C 5C	Coco-Caprylate/Caprate	4.00	Emollient
	DK-PGT Paste Ti (*Daito Kasei Kogyo*)	Polyglyceryl-2 Triisostearate, Titanium Dioxide, Aluminum Hydroxide	3.00	Colorant
D	Chione™ M SVA	Synthetic Fluorphlogopite, Lauroyl Lysine	2.00	Skin feel modifier
	Timica® Terra Yellow MN4502	Mica, Iron Oxides, Titanium Dioxide	1.10	Effect pigment
	Timica® Terra Red MN4506	Mica, Iron Oxides, Titanium Dioxide	0.33	Effect pigment
	Timica® Terra Black MN4498	Mica, Iron Oxides, Titanium Dioxide	0.13	Effect pigment
E	Seanactiv™ BC10113	Aqua, Fucus Vesiculosus Extract, Gluconolactone, Xanthan Gum, Sodium Chloride	1.00	Active ingredient

Specifications:
Viscosity (Brookfield; RVT; spindle TC, Helipath; 20 rpm; 20°C): 17000 mPa s, **Appearance:** Beige fluid

Processing
1: Heat Phases A and B to 80°C under stirring, **2:** Add Phase C into Phase A under stirring, **3:** Add Phase B into Phase A+C under stirring, **4:** Add phase D under stirring, **5:** Cool down to 30°C, then add Phase E under stirring.

Stability:
Stable 3 months at 4°C, RT, 40°C and 45°C

For more information visit us at www.personal-care.basf.com or www.carecreations.basf.com

Silky Eye Concentrate

SC-FR-21-BC-50922-01

BASF
We create chemistry

Enjoy a rested night with the Silky Eye Concentrate, to awake with a fresh look reenergize, free of signs of fatigue and dark circles

Phase	Ingredients	INCI	% by weight	Function
A	Water, demin.	Aqua	84.85	
	Glycerin	Glycerin	3.00	Humectant
	Cosmedia® Ace	Sodium Polyacrylate, Dicaprylyl Carbonate, Polyglyceryl-3 Caprate	1.20	Rheology modifier
	Rheocare® XGN	Xanthan Gum	0.10	Rheology modifier
	Preservative		q.s.	Preservative
B	Lameform® TGI	Polyglyceryl-3 Diisostearate	1.00	Emulsifier (W/O)
	Cetiol® SB 45	Butyrospermum Parkii Butter	1.00	Emollient
	Cegesoft® VP	Olus oil, Hydrogenated Vegetable Oil, Candelilla cera [EU], Vegetable Oil, Hydrogenated Vegetable Oil, Euphorbia Cerifera (Candelilla) Wax	2.00	Emollient
	Cetiol® 4 All	Dipropylheptyl Carbonate	2.50	Emollient
	Preservative		2.50	Emollient
C	Seanactiv™ BC10113	Aqua, Fucus Vesiculosus Extract, Gluconolactone, Xanthan Gum, Sodium Chloride	1.00	Active ingredient
	Perfume	Parfum	0.15	Fragrance
	Sodium Hydroxide (18% solution)	Sodium Hydroxide	0.70	pH Adjustment

Specifications:
pH value (20°C): 5.8, **Viscosity** (Brookfield; RVT; spindle TC, Helipath; 20 rpm; 20°C): 52000 mPa s.

Processing
1: Heat phasesA and B at 75°C, **2:** Add phase B into phase A while mixing, **3:** Allow to cool to room temperature under gentle mixing,
4: Add ingredients of phase C one by one at 30°C.

Stability:
Stable 3 months at 4°C, RT, 40°C and 45°C

Perfume:
Lait de coton RS94159 (TechnicoFlor) (no allergens to declare)

For more information visit us at www.personal-care.basf.com or www.carecreations.basf.com

Azelis Completes the Aquisition of Quimdis

ANTWERP, BELGIUM | 01 SEPT. 2021
Azelis, a leading global innovation service provider in the specialty chemical and food ingredients industry, announces that it has successfully completed the acquisition of Quimdis. Quimdis is a leading French distributor of ingredients for nutraceuticals, flavors & fragrances, animal nutrition, personal care, pharma and food.

Highlights & rationale

- This transaction enhances Azelis' lateral value chain by diversifying and expanding its presence in France and EMEA
- The acquisition of Quimdis provides Azelis in EMEA with an important foothold in the growing non-cyclical markets of flavors and fragrances, synergistic opportunities in animal nutrition, and expands its offering in nutraceuticals
- The transaction is consistent with Azelis' strategy of complementing organic growth with strategic acquisitions

Quimdis was founded in 1988 by Jean-François Quarré, Michel Manalt and Claire Couratier, originally focusing on essential oils and active ingredients. It now specializes in ingredients distribution and formulation development, with solid positions in the French market and with sales into more than 45 countries. Quimdis is headquartered in Paris and has sales close to €100m. Quimdis' 78 employees serve over 1,000 customers and c.400 suppliers. This acquisition strengthens Azelis' offering to fast-growing and attractive markets, whilst also enhancing the position of Azelis France in the life sciences sector. Azelis is now well positioned to develop, structure and implement a global strategy for flavor & fragrance market with the aim of becoming a market leader.

The signing of the deal was announced on July 20th, 2021.

www.azelis.com

SILAB: PEPTILIUM®

The Excellence of Natural Biopeptides for an Anti-aging Effect

SAINT-VIANCE, FRANCE | 31 AUG. 2021
Composed of natural biopeptides purified to 95%, obtained from the co-product of a superfruit, the cranberry, PEPTILIUM® is a global anti-aging active ingredient. Sustainable and effective, it boosts complexion radiance and attenuates fine lines and wrinkles.
As of 21 days of application, PEPTILIUM® tested at 2% in an emulsion significantly improves the parameters characteristic of complexion radiance in Caucasian (reflection: +12%; pink color: +17%) and Asian subjects (luminosity: +6%; olive color: -5%). In the same conditions, PEPTILIUM® presents an anti-wrinkle effect in Caucasian (negative volume: -22%) and Asian (stage of crow's feet wrinkles: -10%) volunteers. These radiance boosting and anti-wrinkle effects intensify after 42 days of treatment and are confirmed by a subjective evaluation: 100% of Caucasian and Asian volunteers consider in particular their fine lines and wrinkles to be attenuated.
A comparative study conducted by SILAB also demonstrates that the efficacy of PEPTILIUM® is more intense and more rapid than that of retinol, while having no side effect.
SILAB got interested in a superfruit, the cranberry (*Vaccinium macrocarpon*). Used by Native Americans in traditional medicine, this small red fruit from North America is today considered to be a health food throughout the world.
PEPTILIUM® (*Vaccinium macrocarpon* (Cranberry) Fruit Extract) is a patented product, available in aqueous solution (recommended amount: 0.5 to 2%). This active ingredient has a content of natural origin of 99% (ISO 16128) and is compliant with international cosmetic regulations (Europe, United States, China, Japan, etc.).

www.silab.fr

BASF and Natural Machines

Partner to Deliver Solutions for Customized Personal Care Face Masks

LUDWIGSHAFEN, GERMANY, AND BARCELONA, SPAIN, 01 SEPT. 2021
BASF and Natural Machines announced their strategic partnership in developing a technology that enables customized face masks and eye patches produced in 3D printers.

Customization of personal care products is a global trend. To meet this growing need, a combination of product expertise and technical understanding is key. BASF, a leading supplier to the personal care industry, and Natural Machines, a solution provider for kitchen and personal care equipment, build upon their respective innovation know-how: a unique 3D printer and 3D printing knowledge from Natural Machines, and the personal care ingredients from BASF. With this new solution users can obtain masks that are not only adaptable to individual face sizes, but also allow the incorporation of different benefits in various zones within the mask.

"*With this partnership we are expanding our personalized cosmetic technologies, and we very much look forward to working with Natural Machines in this promising area. We can build on their experience and expertise of 3D printing to bring this know-how into the personal care market*", says Robert Parker, Director, New Business Development at Care Chemicals, BASF.

"*Our initial tests proved the potential to print face masks and patches based on BASF ingredients. We continue to focus on adapting the technology, to establish a new approach for personalized face masks for our customers based on bio-based and biodegradable solutions*", says Christina Kohlmann, Senior Manager for Open Innovation Personal Care at Care Chemicals, BASF.

"*Our partnership with BASF will help us to grow our portfolio from the food sector to include the cosmetics industry. We will benefit from BASF's experience and latest developments in cosmetic ingredients, while leveraging the broad expertise we have built during the last eight years in 3D food printing. At the same time, BASF's leading position in the personal care market gives us an extraordinary opportunity to commercialize the technology and the device in this space*", says Emilio Sepulveda, CEO of Natural Machines.

BASF and Natural Machines aim to introduce the technology to the market in 2022 globally.

www.basf.com, www.naturalmachines.com.

Experience Beautiful, Radiant "Yoga Skin" with Lubrizol's Oxylance™

Advanced Botanical Ingredient

CLEVELAND, USA | 01 SEPT. 2021
Lubrizol Life Science - Beauty (LLS Beauty) invites consumers to experience glowing, healthier-looking skin, like the radiant look that results from a yoga class, with its new Oxylance™ advanced botanical ingredient.

Oxylance™ advanced botanical ingredient is a botanical extract of Ligustrum lucidum sustainably sourced from the high-altitude mountains of China, where the oxygen levels are low, and extracted through eco-friendly Phenobio™ subcritical water technology. It mimics the Tibetan genetic adaptation to low oxygen conditions, resulting in increased oxygen supply to the skin and overall improved skin appearance.

With age, the skin's microcirculation weakens, leading to impaired oxygenation. These changes can lead to a dull and unhealthy skin appearance. Oxylance™ advanced botanical ingredient combats these signs of aging creating a healthier-looking complexion, while also improving skin oxygen levels, similar to how yoga increases oxygenation to our body.

In vivo study results showed increased skin oxygen levels in a similar way to yoga; more glowing and radiant skin after 28 days; and reduced crow's feet and skin roughness by up to 68% and 32%, respectively. The clinical study also showed improved feelings of happiness similar to a yoga session, and overall healthier-looking complexion for a "yoga-skin" look.

www.Lubrizol.com/beauty

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Phone
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