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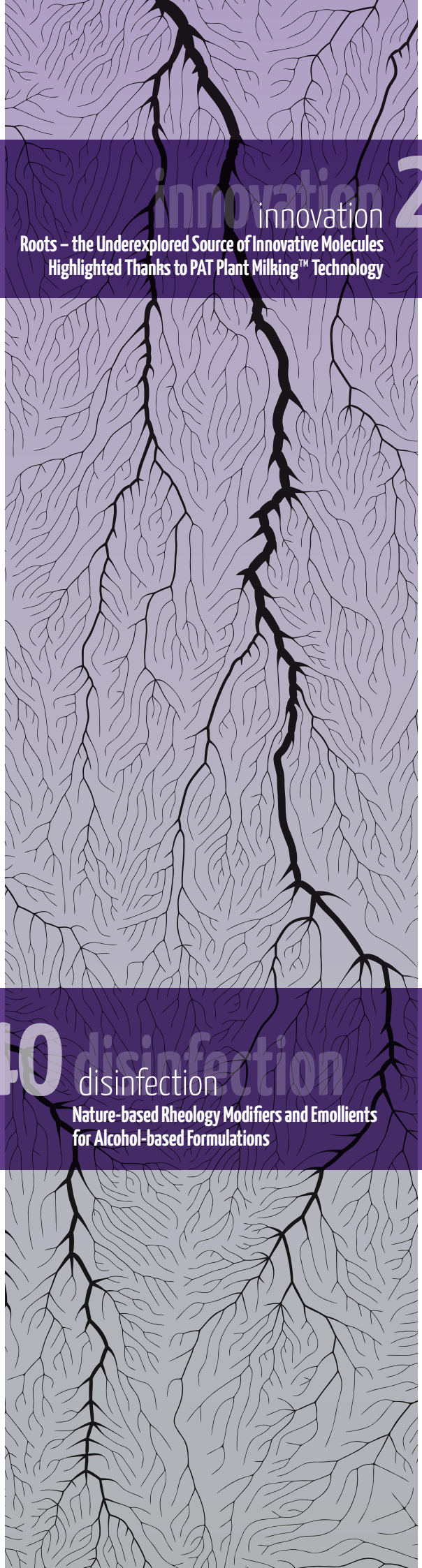

personal care

Roots – the Underexplored Source of Innovative Molecules Highlighted Thanks to PAT Plant Milking™ Technology

A. Salwinski, P. Warnault, A. Guillaumin, L. Duriot, J. Droux, A. Lapeyre, M. Frechet, H. Chajra

abstract

As consumers become increasingly conscious of the need to protect rather than spoil biodiversity on the other side of the world, evolution of technological processes now offers the possibility to get new active molecules from local sourcing. Plant roots, in particular, contain specific and rare compounds with strong biological activities, but were underexplored until recently. A new technology, called Plant Milking™, now allows plant cultivation in aeroponic conditions that give direct access to the roots. Grown in an air environment, they present a different phytochemical profile than those grown in the soil. For the white mulberry tree (*Morus alba*), this corresponds to an enriched content in prenylated polyphenols. As a result, Prenylium™, the extract of *Morus alba* obtained from the Plant Milking™ technology, presents the capacity to protect the skin matrisome against UV radiation and chronoaging, by regulating the expression of COL-3, CCN-1 and MMP-1 genes, whose expressions are dysregulated in aged and photoaged skins. In addition, the synergistic effect of these prenylated polyphenols in Prenylium™ has a stronger collagenase inhibition than the molecules taken individually, demonstrating the benefit of using such an extract. Thanks to these biological activities, Prenylium™ clinically demonstrates a significant capacity to reduce wrinkle depth, improve skin smoothness and promote skin plumping.

Introduction

Recent changes in the world make consumers even more concerned about the products they buy and use. As the realization that we can't continue to spoil biodiversity grows among consumers, they are looking increasingly for safer products with high quality from thoughtful sourcing. While in the cosmetic industry many of the latest active ingredients come from exotic or deep-sea origins, there is also an underexplored source of highly performing molecules right under our feet that we could be using to create new active ingredients: plant roots. As roots grow in the ground, a harsh environment where they are subjected to a large variety of aggressions (both mechanical because of the minerals present in the soil, and biological due to micro-organisms), they have developed specific defense mechanisms. One is the production of secondary metabolites that are sometimes exclusive to this plant part or found in richer quantities than in other parts. These metabolites possess biological activities that have been used for centuries by humans, through traditional medicine and more recently to create drugs. But to get access to them, it was necessary to uproot the plant from the soil, which lead to its irreversible destruction. Therefore, only roots from a limited number of plants could be used, as it is not possible to take those of trees or rare species. In addition, industrial production of plant root extracts was not possible as the production of secondary metabolites by roots was inconsistent, due to modifications of their environment from one year to the next and the risk of extracts being contaminated by external substances coming from the soil.

All these reasons explain why this source remained underexplored until now. Fortunately, thanks to the development of an innovative technological process called Plant Milking™, it has become possible to access roots in a sustainable and controlled way. This paper presents the principle of this technology and its application in the development of an innovative cosmetic active ingredient extract with a unique phytochemical profile. The plant extract discussed in this paper comes from a tree called *Morus alba* commonly known as white Mulberry. This tree belongs to the Moraceae family. It is a rounded, fast-growing and deciduous tree that can grow up to 20 meters high. Native to China, it has long been used in Asia and Europe for silk production. Valued for its therapeutic properties, mulberry is now widely distributed all around the globe [1, 2]. One part of the tree however still has potential to reveal: the wide and spreading root system. So far, it was challenging to study this organ as it meant destroying the plant, making this tree a good candidate for PAT Plant Milking™ technology. The principle of this technology [3] resides in growing the plants in aeroponic conditions, which means without soil and the roots are directly accessible. They can be cut and regrow naturally, thus respecting the life cycle of the plant and allowing multiple harvests per year. Nutritive solutions are sprayed on the roots, adjusted for each plant species, to stimulate the production of targeted secondary metabolites. These conditions ensure an increased yield of phytocompounds compared to that found in nature. In addition, the plants are cultivated in greenhouses, which means

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their environment is kept under control to ensure regular production from one harvest to the next. Irrigation systems are optimized, and the water is recycled to reduce its consumption. Furthermore, there is no use of pesticides or fertilizers that may pollute the soil and the underground water. All these features make the Plant Milking™ technology highly sustainable and eco-friendly.

Results and Discussion

Plant cultivation performance

Three-year-old *M. alba* trees cultivated in aeroponic conditions produced a prolific biomass for both roots and aerial parts (**Fig. 1**). Thin roots were abundant and presented a pronounced yellow color suggesting a high content in flavonoids (**Fig. 2**).

Phytochemical profiles of *Morus alba* root extracts according to their culture conditions

The phytochemical profiles of *Morus alba* root extracts revealed the presence of prenylated polyphenols. Interestingly, the type and the quantities of these prenylated polyphenols varied according to plant culture conditions. Indeed, when the plant was cultivated in soil (commercial roots) few prenylated compounds were found (**Fig. 3C** and **Tab. 2**, Moracenin B; Moracenin A and Kuwanon C). In contrast, when the plant was cultivated under aeroponic conditions either stimulated or not, new prenylated polyphenols were produced (**Fig. 3A** and **B**, and **Tab. 1**). We identified in addition the Wittiorumin F and Mulberrofuran T. Interestingly, we found that if we deprived the plant of nitrogen, we could enrich the root's content in prenylated polyphenols (**Fig. 3B** and **Tab. 1**). This demonstrates the potential of the Plant Milking™ technology for sourcing rare and original molecules from commonly used plants.

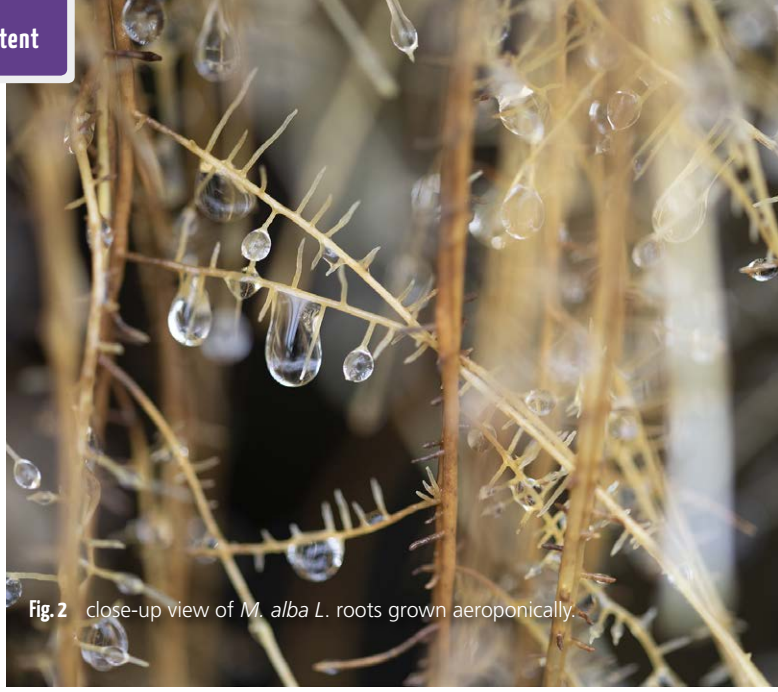

Fig. 2 close-up view of *M. alba L.* roots grown aeroponically.

Prenylium™ protects dermal fibroblasts and skin matrisome against UV damage

Solar ultraviolet (UV) radiation is the most important environmental factor contributing to photoaging [4]. Among UV radiation spectrum, UVB are known to be responsible for skin damage due to ROS accumulation, DNA alteration and pro-inflammatory cytokines production and release [5].

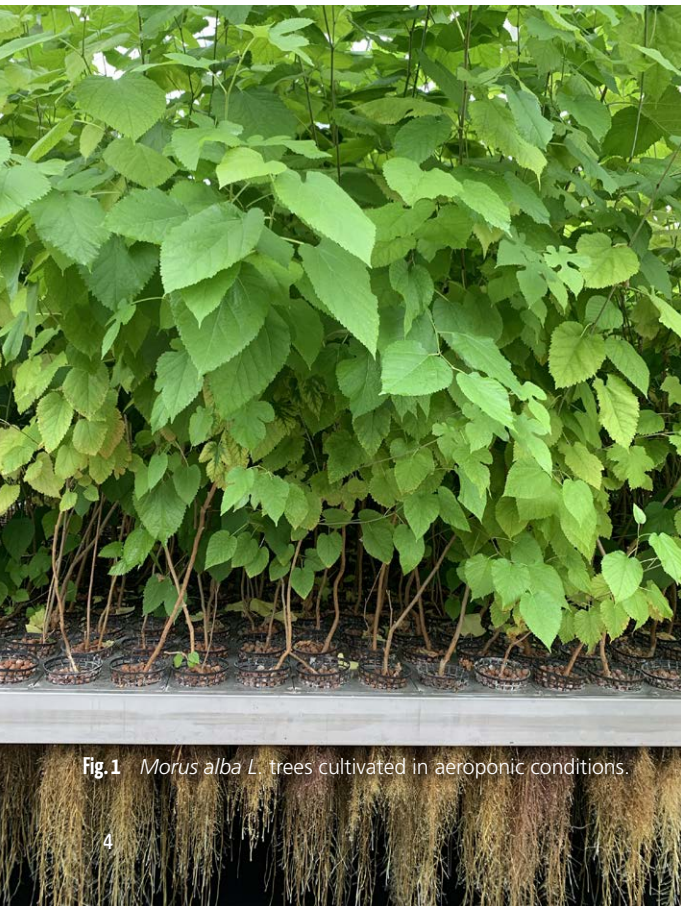

Fig. 1 *Morus alba L.* trees cultivated in aeroponic conditions.

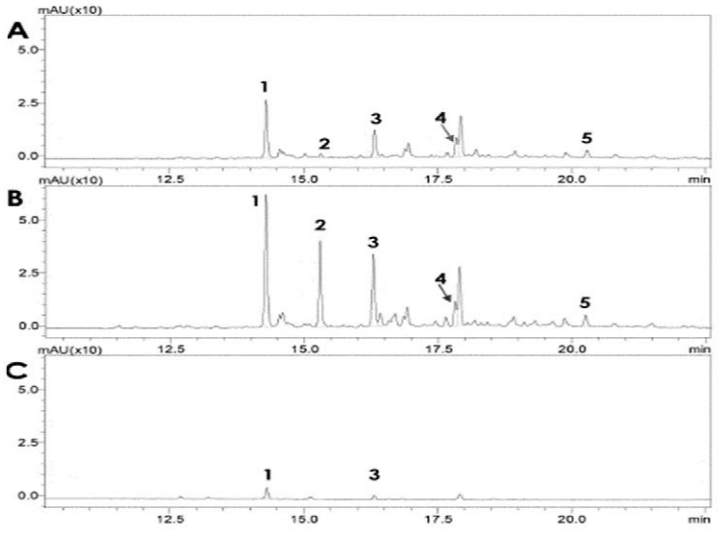

Fig. 3 Phytochemical profiles of *Morus alba* root extracts: UHPLC chromatograms of the extracts obtained from roots cultivated in aeroponic system without (A) and with plant stimulation with nitrogen deficiency (B) and from commercially available roots (C). The metabolites of *Morus alba* root extracts were identified using the mass spectrometry technic. The following numbers refer to the following compounds: 1. Moracenin B, 2. Kuwanon C, 3. Moracenin A, 4. Morusin and Wittiorumin F, 5. Mulberrofurane T.

The epidermis and dermis are both affected by UVB. In dermis, it has been demonstrated that matrisome genes referring to two main groups of proteins called core matrisome group (collagens, proteoglycans, glycoproteins and matrisome associated genes are also dramatically affected by UVB irradiation [6, 7]. The core matrisome group include collagens, proteoglycans and glycoproteins and the matrisome associated genes refers to syndecans, enzymes (such as MMPs, transglutaminases) and their inhibitors and secreted factors (cytokines). Several studies have demonstrated that collagen type I decreases in photoaged skin due to increased collagen degradation but also due to the failure to replace damaged collagen with newly synthesized collagen type I and type III by dermal fibroblasts [8-10]. Various matrix metalloproteinases (MMPs) participate in matrisome dermis degradation [11]. The direct consequences of the degradation of the collagenous matrix and dermis matrisome by MMPs, transiently up-regulated during UVB irradiation, and the limited fibroblast capabilities to induce the renewal of collagen type I and type III, lead to wrinkle occurrence.
Interestingly, we found that Prenylium™ regulates the mRNA expression of collagen type III (COL 3) and cysteine rich angiogenic protein 61 (CCN-1) (**Fig. 4A**) in human dermal fibroblasts. Both COL 3 and CCN-1 are dysregulated in aged and photoaged skins. Moreover, in 2013, Qin *et al* demonstrated that imbalanced collagen homeostasis is mediated in part by elevated expression of CCN-1 in dermal fibroblasts exposed to UV irradiation [12].

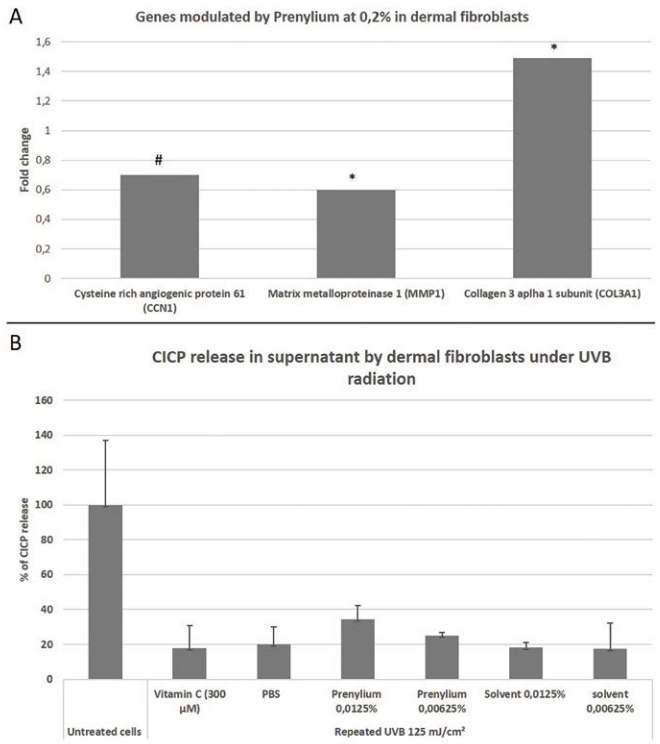

Fig. 4 A. Genes modulated in fibroblasts when treated for 24h with Prenylium™ at 0.2%. The relative expression (RQ) of each gene was calculated in comparison to fibroblasts treated with the vehicle DMSO at 1% (RT-QPCR, statistical analysis student t test # $p < 0.1$ and *$p< 0.05$). B. Quantification of CICP secreted in the supernatant of normal human fibroblasts NHDFs under various conditions. (Elisa assay).

Compound	Content in Moracenin B equivalent for 20 mg of milled and dried roots (mg/L)		% of increase in comparison to non stimulated condition
	Morus alba root extract cultuvated in aeroponic condition		
	Stimulated condition	Non stimulated condition	
Moracenin B	126.1 ± 3.1	53.5 ± 1.2	**136**
Moracenin A	73.8 ±1.1	28.5 ± 0.7	**159**
Kuwanone C	83.2 ± 1.6	3.4 ± 0.1	**2358**
Wittiorumine F	22.8 ± 0.6	18.1 ± 0.7	**26**
Mulberrofurane T	13 ± 0.5	9.3 ± 0.4	**39**

Tab. 1 Root extract content in each prenylated polyphenol identified according to plant culture conditions. The content is expressed in Moracenin B equivalent (mg/L).

Compound	Content in Moracenin B equivalent for 20 mg of milled and dried roots (mg/L)		% of increase in comparison to commercial root
	Morus alba root extract		
	Stimulated under aeroponic condition	Traditional culture condition (in soil)	
Moracenin B	126.1 ± 3.1	11.74 ± 0.34	974
Moracenin A	73.8 ±1.1	0.16 ± 0.04	46025
Kuwanone C	83.2 ± 1.6	3.86 ± 0.22	2055
Wittiorumine F	22.8 ± 0.6	0	NA
Mulberrofurane T	13 ± 0.5	0	NA

Tab. 2 Root extract content in each prenylated polyphenol identified according to plant culture conditions. The content is expressed in Moracenin B equivalent (mg/L).

They demonstrated also that CCN-1 is strongly elevated in sun exposed prematurely aged skin and in aged cells [13]. They demonstrated also that elevated concentration of CCN-1 rapidly inhibits type I procollagen production and upregulates matrix metalloproteinases (MMP-1, 3 and 9: collagenases known for their capability to degrade collagen fibrils) [13]. It appears clear that protecting skin matrisome against external aggressions such as UV, pollution or oxidative stress is a way to limit skin declines that appear with aging, thus limiting wrinkles' apparitions, skin thinning, loss of skin elasticity and firmness. Therefore, finding a plant extract offering the capability to mitigate CCN-1 expression, such Prenylium™ does, might be an efficient solution for fighting premature aging. Additionally, we demonstrated that Prenylium™ also significantly inhibits MMP-1 expression, revealing its anti-aging property.

Then, we exposed human fibroblasts to UVB radiation in the presence or not of Prenylium™ in order to assess the impact of this deleterious stress on an essential matrix protein, collagen type I. We quantified CICP procollagen type I C propeptide released in the supernatant. As expected, when skin fibroblasts were irradiated by UVB the procollagen synthesis cell capabilities were decreased [14]. CICP concentration measured in the UVB stressed fibroblasts' supernatant was dramatically reduced by -70% (**Fig. 4B**). We observed that when fibroblasts were pretreated by Prenylium™, this dramatic effect was mitigated. The benefit provided is dose dependent. Indeed, in comparison to non-stressed condition, the concentration of CICP released is decreased only by -62% and -48% respectively for the following Prenylium™ concentrations 0.00625% and 0.0125%. When we compared the data with the stressed condition and without Prenylium™, we found an improvement in CICP release by +21% and +71% respectively. These results suggest that the ability of the cells to produce and release CICP in the supernatant even though highly impacted by the repeated UVB irradiation, is still functional. The solvent used to dilute Prenylium™ was also tested and didn't show any effect on the improvement observed. Moreover, Vitamin C, in our experimental conditions is not efficient for preserving fibroblasts synthesis ability. Our data demonstrate a protective effect of Prenylium™ regarding fibroblasts' abilities to ensure CICP production and release under UVB stress.

Prenylium™ inhibits collagenase activity

Skin metalloproteinases such as collagenases, gelatinases, stromelysins and matrilysins are major enzymatic mediators of premature skin aging induced by sun UV radiation [11]. Indeed, these enzymes degrade skin extracellular matrix, accelerating premature skin aging. Among these enzymes, the collagenase MMP-1 degrades collagen type I, the principal dermal protein found responsible for skin firmness and mechanical properties. In addition to its role in the maintenance of collagen type I production by fibroblasts exposed to UVB, we addressed the impact of Prenylium™ on collagenase activity, asking the question of whether it also protects this newly synthetized collagen from degradation. For this purpose, a functional collagenase enzymatic evaluation was set up. This enzyme cleaves the synthetic peptide FALGPA into GPA and FAL. The release of FAL was then determined by HPLC thus allowing the calculation of collagenase activity (AcTR). Interestingly, we observed that Prenylium™ had a higher collagenase inhibitory effect (-73%) in comparison to each prenylated molecule tested at the same concentration (Moracenin B: -42%, Kuwanone C: -38%, Morusine: -32% and Moracenin A: -62%). Our results suggest a synergic effect of these prenylated compounds contained in Prenylium™ (**Fig. 5**). Then, we compared the collagenase inhibitory effect of Prenylium™ to the traditional root extract, in order to determine if this inhibitory effect is provided by the unique Prenylium™ composition. In contrast, we have shown that traditional root extract, which means the extract is obtained from a *Morus Alba* tree cultivated in soil, demonstrates no inhibitory effect regarding to collagenase activity.

Prenylium™ reduces wrinkle depth, improves skin smoothness and skin plumping

As wrinkled skin appearance and rough textured skin are common criteria of photoaged skins [10] easily visible in the crow's feet area, we focused our investigation of the clinical efficacy of Prenylium™ on its capability to improve these kind of wrinkles. The volunteers were asked to apply for 2 months, twice daily, the cosmetic product at 1% half face and the placebo cream half face. After 1 and 2 months of Prenylium™ use, the analysis of crow's feet wrinkles with C-Cube shows a statistically significant improvement of skin relief parameters Sv (which represents the deepest wrinkles) and Sz (which represents skin smoothness). After 1 and 2 months, in comparison to the placebo group, with Prenylium™ the Sv pa-

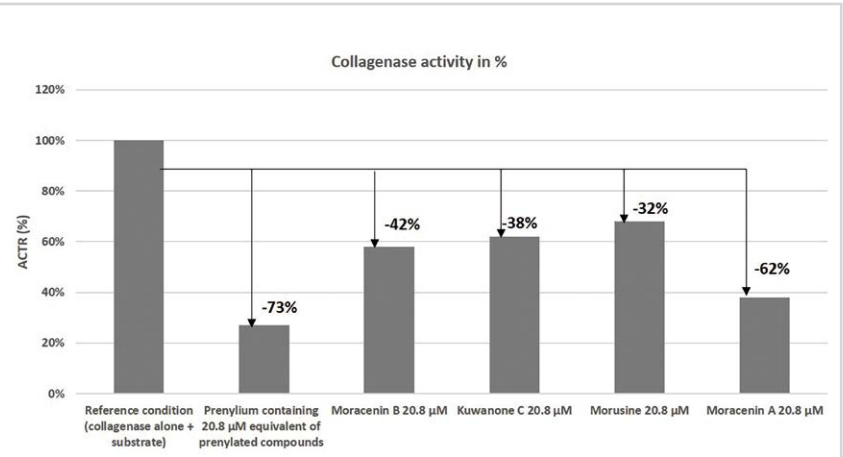

Fig. 5 Evaluation of collagenase inhibitory activity of *Morus alba* extract and its prenylated compound using HPLC technique

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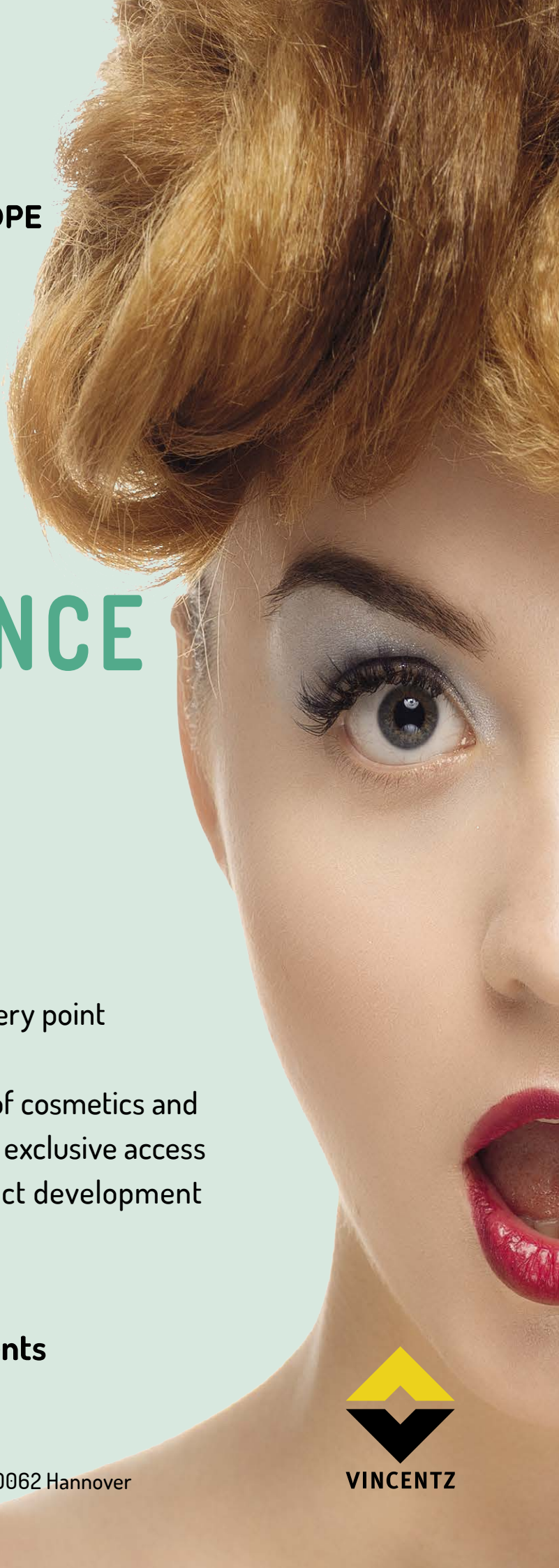

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rameter was reduced by -12.7% and -16.6% (**Fig. 6A**) while Sz parameter was reduced by -15.4% and -19.9% (**Fig. 6B**). We observed also a time dependent improvement effect for these two parameters for the Prenylium™ group in contrast to the placebo group where an increase was observed. These results were confirmed in heat maps. Indeed, in the pre-treatment heat map, blue, purple and red represented the depth of wrinkles. One and two months after Prenylium™ treatment, these strong colors had been replaced with green and yellow, demonstrating an improvement in skin wrinkles' depths (**Fig. 7**). The skin plumping determined by a 10-grade scoring scale was significantly improved compared to the placebo (**Fig. 6C**). In addition, we noted that the plumping effect observed is time dependent. Indeed, when considering Prenylium™ formula, skin plumping effect was significantly higher after 1 month and even greater after 2 months compared to D0 (+5.6% and +13.4% respectively).

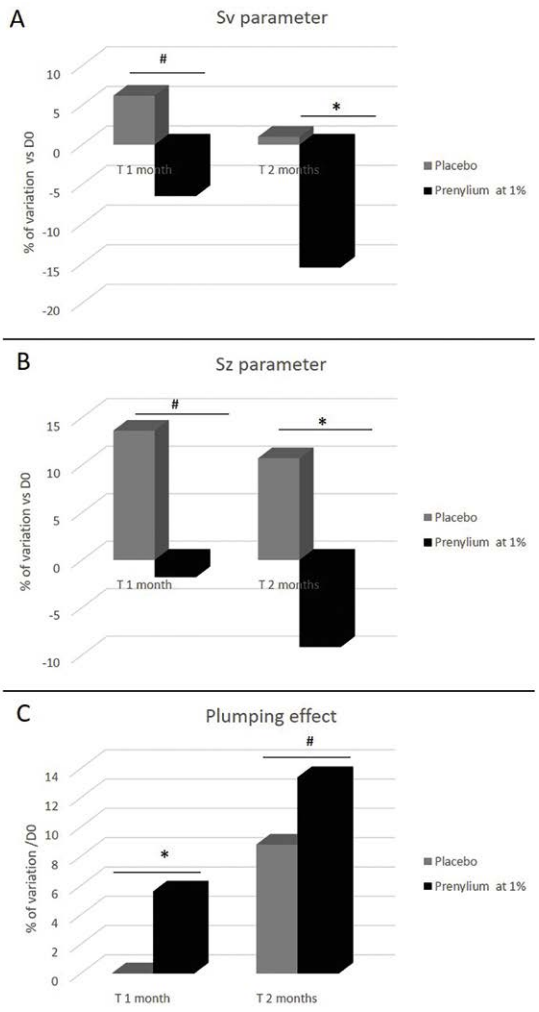

Fig. 6 Clinical measurement of skin wrinkles' depth (sv parameter), skin smoothness (sz parameter) and skin plumping effect before and after 1 and 2 months of a cosmetic cream application containing either placebo or Prenylium™ (1%) on the face. Sv and Sz parameters determined with C cube and skin plumping effect determined by the use of a grading scale. The range of grading scale varies from 0 (sagging, skin not firm) to 10 (tonic, firm and plumped skin). The measurements were done on crow's feet area.

Conclusion

We demonstrated that the Plant Milking™ technology applied to a tree, the *Morus Alba L*, allows the production of a unique plant extract highly enriched in prenylated compounds, which is not comparable to a commercial root extract, and harboring strong anti-aging biological properties. Indeed, we demonstrated that this enriched plant extract protects human dermal fibroblasts against UV damages, by regulating the expression of CCN-1, collagen type III and MMP-1 while maintaining procollagen type I synthesis and release. These biological molecules are all deregulated by sun UV radiation but also during chronological aging. Moreover, we demonstrated that the biological activity discovered, related to the inhibition of collagenase activity, is only found in the enriched extract and not in the extract prepared from a commercial root cultivated in soil. Finally, we confirmed the anti-aging biological properties found in vitro at the clinical level. Indeed, after 2 months of Prenylium™ use, less visible wrinkles, and a smoother and plumped skin were demonstrated. This experimental work highlights Plant Milking™ technology as an unrivaled sustainable solution for the discovery of innovative active ingredients enriched in active molecules, such as prenylated flavonoids with unique biological properties which will meet demanding customer-related sustainability expectations.

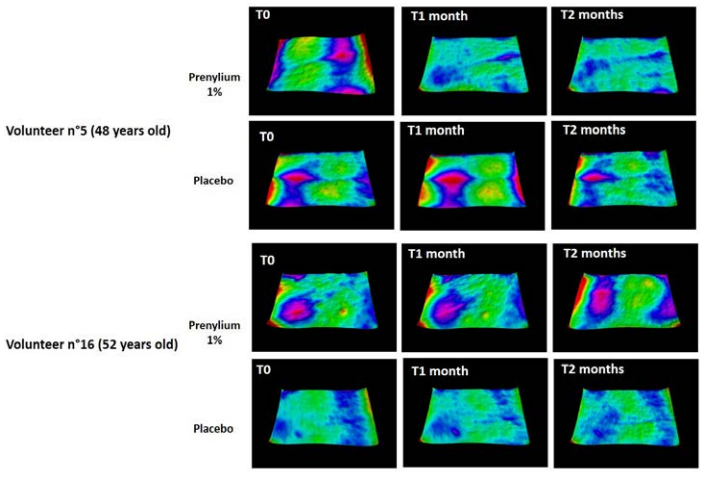

Fig. 7 3D Heat maps representing wrinkles before and after 1 and 2 months of product use. The pictures represented crow's feet area. In the heat map, dark blue, purple and red colors represent depth wrinkles.

Materials and Methods

Morus alba plant culture and root extracts preparation

The seeds of *Morus alba* were purchased from a French supplier (Les Semences du Puy, Le Puy-en-Velay, France). Then, we preserved and propagated the *Morus alba* plants in our greenhouse. *Morus alba*

plants were cultivated aeroponically for 8 weeks in a 15/10/30 (N/P/K) culture medium, then in the case of stimulated plants for 3 weeks in a nutrient solution deprived of nitrogen. The extraction process was conducted in the same way for all root samples. Briefly, dry roots were grounded and extracted with pure ethanol (20 mg of ground roots/mL) by vortexing at room temperature. The *Morus alba* root extract corresponding to stimulated condition (deprived of nitrogen) corresponds to Prenylium™ product. All experiments were done in triplicate.

Determination of phytochemical profiles of *Morus alba* root extracts

The phytochemical profiles of all *Morus alba* root extracts were determined using UHPLC Shimadzu (Shimadzu, Kyoto, Japan) coupled to a mass spectrometer LCMS2020 (electrospray ionization in a negative ion mode, m/z 200-1000). The concentration of each component in mg/L was calculated by using Moracenin B as a standard.

Procollagen type I C Propeptide (CICP) quantification

Normal human foreskin dermal fibroblasts (NHDFs) were seeded in 24 well plates and cultivated in DMEM 1% fetal bovine serum (Gibco, ref 10270-106, Thermofisher scientific) supplemented with penicillin/streptomycin (Gibco, ref 15140-122, Thermofisher scientific) for 72h. Then, the culture medium was replaced by a fresh DMEM medium containing Prenylium™ at 0.00625% and 0.0125% or the corresponding solvent (1.3 propanediol at 80%) at the same concentrations for 24 hours before the first UVB irradiation (125mJ/cm^2). The DMEM medium was renewed each day with Prenylium™ or the solvent before each UVB irradiation. NHDFs were UVB irradiated once per day for 4 days. Then, the supernatants were collected for the dosage of procollagen type I C propeptide (CICP). CICP concentration was assessed by ELISA assay according to manufacturer instructions (CICP microvue Quidel kit, ref 8003, Tecomedical). The CICP concentrations measured were normalized to MTS viability data. The cell viability was determined with a MTS (3-4,5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolim) assay (Promega, ref G3581). The experiment was done in triplicate.

Gene expressions study

NHDFs (ATCC, reference CRL-2522, LGC Promochem). Prenylium™ at 0.2% was applied on fibroblasts for 24 hours (in triplicate). Transforming growth factor-β1 at 20 ng/mL was used as positive control. After treatment, total RNAs were extracted using the RNeasy Mini Kit (Ref. 74106) from Qiagen (Hilden, Germany) according to the manufacturer's instructions. Then the extracted RNAs were quantified (Ultrospec 1100 Pro-Amersham) and their integrity was analyzed by cap-

illary electrophoresis (Agilent Bioanalyzer 2100 – Agilent RNA 6000Nano Kit, 5067-1511). For specific genes targeting matrisome related genes, gene expression levels were quantified by qRT-PCR using TaqMan® cards from Applied Biosystems (Carlsbad, CA, USA). The following matrisome related genes were studied: Col3A1 (assay ID-Hs00943809_m1), Cyr61-(assay ID Hs00998500_g1) and MMP-1 (assay ID Hs00899658_m1). Normalization method used was based on the reference to a housekeeping gene, ie, glyceraldehyde-3-phosphate dehydrogenase (assay ID Hs02758991_g1). Data Assist Software v3.01 was used for data analysis. DataAssist™ Software is a simple, yet powerful data analysis tool for sample comparison when using the comparative CT (ΔΔCT) method for calculating relative quantitation of gene expression. It contains a filtering procedure for outlier removal, various normalization methods based on single or multiple genes, and provides relative quantification analysis of gene expression through a combination of statistical analysis and interactive visualization. A two-sample, two-tailed Student's t-test comparing the ΔCT values of the two groups is performed, and a p-value was calculated.

Collagenase inhibition activity

The principle of the evaluation is based on the known catalytic hydrolysis activity of a synthetic peptide FALGPA (N-[3-(2-Furyl) acryloyl]-Leu-Gly-Pro-Ala) into GPA (Gly-Pro-Ala) and FAL (N-[3-(2-Furyl)acryloyl]-Leu) by the collagenase. To determine the rate of enzymatic conversion, the content of FAL is determined by UHPLC in presence of test sample or in its absence. A collagenase assay kit was used following manufacturer's instruction.

Clinical investigation

This randomized double blind, placebo controlled clinical trial was conducted in France by Dermatec (France, Grenoble). The study was conducted in accordance with the principle of the Declaration of Helsinki and the guidelines of the International Conference on harmonization Good Clinical Practice as applicable to a non-drug study. The volunteers gave written and informed consent. Twenty-two Caucasian females who gave their informed consent were enrolled in this clinical study. The study duration was 8 weeks with an intermediate time point at 4 weeks. The volunteers were in the age range from 45 to 70 years, were presenting wrinkles, and a dull and uneven complexion. Subjects applied the placebo and the product containing the active ingredient Prenylium™ at 1% twice a day on their half face. The formula's used were the following (see **Formula**). The parameters measured with a dermatoscope C-cube, a device that takes high 2D and 3D resolution pictures, were wrinkle depth (sv) and skin smoothness (sz). While Sv measures the valley depth, revealing the deepest wrinkles, Sz represents the total amplitude (difference between highest peak and deepest valley), correlated with skin smoothness. The plumping effect of the product was also determined using 11 points scoring scale. A 0 score means sagging skin and not firm, while 10 score means a tonic skin, firm and plumped up. The plumping effect is assessed visually by a trained assessor.

Conflict of interest

The authors state no conflict of interest.

References

[1] *Nomura T, Hano Y, Fukai T.* Chemistry and biosynthesis of isoprenylated flavonoids from Japanese mulberry tree. Proc Jpn Acad Ser B Phys Biol Sci. 2009;85(9):391-408.

[2] *Wei H, Zhu J-J, Liu X-Q, Feng W-H, Wang Z-M, Yan L-H, et al.* Review of bioactive compounds from root barks of Morus plants (Sang-Bai-Pi) and their pharmacological effects. Cogent Chemistry. 2016;2(1).

[3] *Gontier E, Clement A, Tran TLM, Gravot A, Lièvre K, Guckert A, et al.* Hydroponic combined with natural or forced root permeabilization : a promising technique for plant secondary metabolite production. Plant science. 2002;163:723-32.

[4] *Rittie L, Fisher GJ.* Natural and sun-induced aging of human skin. Cold Spring Harb Perspect Med. 2015;5(1):a015370.

[5] *Khalil C.* Human skin explants an *in vitro* approach for assessing UVB induced damage. Toxicol *In Vitro*. 2018;53:193-9.

[6] *Hynes RO, Naba A.* Overview of the matrisome – an inventory of extracellular matrix constituents and functions. Cold Spring Harb Perspect Biol. 2012;4(1):a004903.

[7] *Naba A, Clauser KR, Ding H, Whittaker CA, Carr SA, Hynes RO.* The extracellular matrix: Tools and insights for the „omics" era. Matrix Biol. 2016;49:10-24.

[8] *Varani J, Spearman D, Perone P, Fliegiel SEJ, Datta S, Quan Wang Z, et al.* Inhibition of type U procollagen synthesis by damaged collagen in photoaged skin and by collagenase degraded collagen *in vitro*. American Journal of Pathology. 2001;158(3):931-.

[9] *Zhang S, Duan E.* Fighting against Skin Aging: The Way from Bench to Bedside. Cell Transplant. 2018;27(5):729-38.

[10] *Poon F, Kang S, Chien AL.* Mechanisms and treatments of photoaging. Photodermatology, photoimmunology & photomedicine. 2015;31(2):65-74.

[11] *Pittayapruek P, Meephansan J, Prapapan O, Komine M, Ohtsuki M.* Role of Matrix Metalloproteinases in Photoaging and Photocarcinogenesis. International journal of molecular sciences. 2016;17(6).

[12] *Qin Z, Okubo T, Voorhees JJ, Fisher GJ, Quan T.* Elevated cysteine-rich protein 61 (CCN-1) promotes skin aging via upregulation of IL-1beta in chronically sun-exposed human skin. Age (Dordr). 2014;36(1):353-64.

[13] *Quan T, Qin Z, Robichaud P, Voorhees JJ, Fisher GJ.* CCN-1 contributes to skin connective tissue aging by inducing age-associated secretory phenotype in human skin dermal fibroblasts. J Cell Commun Signal. 2011;5(3):201-7.

[14] *Qin Z, Robichaud P, He T, Fisher GJ, Voorhees JJ, Quan T.* Oxidant exposure induces cysteine-rich protein 61 (CCN-1) via c-Jun/AP-1 to reduce collagen expression in human dermal fibroblasts. PloS one. 2014;9(12):e115402.

Ingredients	Quantity (%) Prenylium™ formula	Quantity (%) placebo formula
Water	q.s	q.s
Glycerin	3.00	3.00
Glyceryl stearate (and) PEG-100 stearate	3.00	3.00
Isopropyl myristate	3.00	3.00
Cyclopentasiloxane	3.00	3.00
Cetyl alcohol	2.50	2.50
Cyclopentasiloxane (and) Dimethiconol	2.00	2.00
Prenylium™	**1.00**	–
Water (and) Sodium hydroxide	0.60	0.60
Phenoxyethanol	0.50	0.50
Ethylhexylglycerin (and) Tocopherol	0.40	0.40
Chlorphenesin	0.20	0.20
Carbomer	0.20	0.20
Perfume (fragrance)	0.10	0.10

Formula

contact

Aleksander Salwinski, Pierre Warnault, Agnès Guillaumin, Léonor Duriot

Plant Advanced Technologies
PAT, 19, Avenue Forêt de Haye, 54500 Vandoeuvre, France

Julie Droux, Alexandre Lapeyre, Mathilde Frechet, Hanane Chajra

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Ingestible Beauty

S. Schulze

There are strong market opportunities for natural, science-backed multifunctional ingredients in the growing beauty-from-within segment.

The body's largest organ, the skin, is one that requires a lot of nourishment and care. Topical skin care applications still have their appeal, but ingestible beauty products are on the fast track. More than ever, the link between nutrition and skin conditions is obvious to consumers. If you provide your body with the right nutrients and create a healthy internal environment, it will show on the outside. Increasing interest from younger consumers keen to preserve their natural youth is a major driver behind the category's growth. Similarly, the ever-growing ageing population wants to retain a youthful appearance into later life, thus contributing to the demand for high quality beauty ingredients. According to Innova Market Insights, the key trends in new product development are evidence of efficacy, naturality of ingredients and their ability to be multifunctional. Clearly, this creates opportunities for many ingredients. The following article will focus on three main targets for healthy skin: antioxidant protection, elasticity and moisture retention.

The Antioxidant Power of Astaxanthin

The skin consists of three main layers: the epidermis, the dermis and the hypodermis. Topical applications like serums and creams are able to influence the surface of the skin – the epidermis – but they can't get down into the deeper layers, which contribute to skin elasticity and nourishment. However, research shows that natural astaxanthin is able to act on all of these layers and promote healthy skin from the inside out. Obtained from the microalga *Haematococcus pluvialis* (**Fig. 1**), the antioxidant improves the supply of nutrients to the hypodermis, protects collagen integrity in the dermis, neutralises the UV-induced free radicals that cause skin damage in the epidermis and prevents roughness by improving moisture retention in the skin's outermost layer – the *stratum corneum*.

Clinical trials have confirmed that AstaReal® natural astaxanthin prevents skin deterioration during the seasonal changes that occur between August and December, when environmental factors such as UV light and varying degrees of moisture in the air tend to exacerbate skin problems [1]. In one trial, while wrinkles and dryness worsened in the control group, the astaxanthin group was more resilient and maintained their healthy skin throughout the seasons. This result suggests that long-term prophylactic astaxanthin supplementation may inhibit age-related skin deterioration, thanks to its antioxidant and anti-inflammatory effects.

Protecting Underlying Structures

As we age, we produce less of the proteins naturally found in skin, like collagen, hyaluronic acid and fibrin. The external effects of these losses are wrinkles, fine lines and dryness. In addition, UV-induced free radical damage causes wrinkles by degrading collagen and hyaluronic acid. However, it is possible to slow these processes down by providing targeted nutritional supplements. A study in which 28 women took 6 mg natural astaxanthin for eight weeks in combination with topical treatment showed improvements in skin wrinkles, age spot size, elasticity, texture and moisture content of the skin [2]. Mean depth and width of the wrinkles around the eye region were reduced compared to the measurements before the treatment. Astaxanthin is able to quench free radicals in the skin and thus protect collagen fibrils from accelerated breakdown.

Age is still the most important factor when fulfilling consumers' skin health needs. According to Innova Market Insights, 5 out of the top 10 positionings for new skin care products during 2019 were "ageing well" and skin health claims [3]. Consumers are looking for ways to prevent signs of ageing while also supporting the underlying health of their skin. As ageing is closely associated with the loss of collagen and hyaluronic acid, and the deterioration of connective tissue, supplementation with bioactive collagen peptides and hyaluronic acid helps to counteract the consequences of these natural processes.

Collagen Peptides: The Secret Beauty Boosters

Essential for the skin's firm structure, collagen is the most abundant protein in the body and a key constituent of all connec-

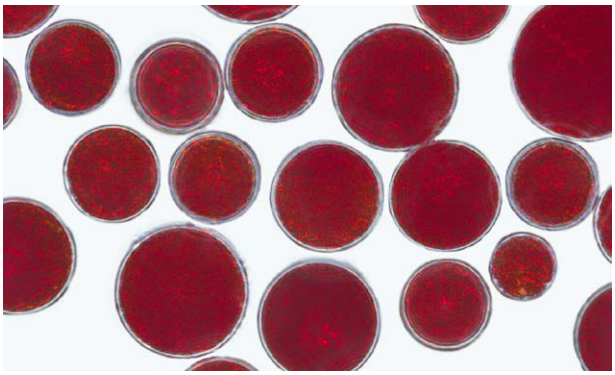

Fig. 1 *Haematococcus pluvialis algae* under the microscopy (©AstaReal).

tive tissues. Specifically optimised Bioactive Collagen Peptides® from Gelita are able to stimulate the fibroblasts in the dermis upon supplementation. This substantially increases the synthesis of collagen, elastin and proteoglycans, all of which are essential for providing elasticity. As a result, the epidermis is supported from beneath and skin sagging is prevented. (**Fig. 2**)

The effects of Gelita's VERISOL® collagen peptides on epidermal and dermal skin structures have been scientifically confirmed in three placebo-controlled human clinical studies. An initial study verified the effectiveness of collagen peptides on biophysical skin parameters related to cutaneous ageing [4]. In the double-blind, placebo-controlled trial, 69 women aged 35-55 were randomised to receive 2.5 g or 5.0 g of VERISOL® or a placebo orally once a day for 8 weeks. After only four weeks of treatment, skin elasticity in both treatment groups showed a statistically significant improvement compared with the placebo group. Interestingly, the positive impact of collagen peptide treatment on skin elasticity was more pronounced in women aged 50 and older. The positive impact was still detectable at the end of the 4-week washout phase, suggesting a long-lasting dermal physiological effect. A clinical follow-up study demonstrated the effectiveness of collagen peptides on wrinkle reduction and the synthesis of important dermal matrix components such as type I collagen, elastin and fibrillin [5]. Beyond wrinkle reduction, VERISOL® is also able to treat cellulite in normal and overweight women [6].

Maintaining Moisture with Hyaluronic Acid

Hyaluronic acid is another core component of skin and its extracellular matrix. Roughly half of the hyaluronic acid in the body is present in the skin, where it binds to water to help retain moisture. Together with collagen, it lays the foundation of the skin's elasticity and smooth appearance. Hyaluronan has critical moisturising properties and a high water retention capacity, which makes skin appear smoother. Doses of 120-240 mg per day for at least one month have been shown to significantly increase skin moisture and reduce dry skin in adults [7]. Hydrated skin also reduces the appearance of wrinkles, as shown in a double-blind placebo-controlled study with 60 male and female subjects aged 22-59 years who presented with crow's feet wrinkles [8]. Participants took 120 mg hyaluronic acid per day or a placebo for 12 weeks, with results suggesting that oral hyaluronic acid inhibits wrinkles and improves skin condition.

Emerging Evidence

Classical ingredients such as vitamin C and zinc also remain very popular, supported by their traditional uses and European Food Safety Authority (EFSA) claims in Europe. Beyond that, some phytonutrients like lycopene are well established in sun-related skin care formulations. In addition, one of the latest trends is probiotics and prebiotics. Consumers are now aware of the link between a healthy microbiota and its benefits for the skin, as emerging science shows evidence on the subject.

Sometimes, the best effects can be achieved by combining several nutrients. For instance, in a placebo-controlled study, researchers found that women taking an astaxanthin-collagen combination had significantly improved skin elasticity and skin barrier integrity, resulting in reduced moisture loss and a lower expression of collagen and elastin degrading enzymes [9].

Market Domination for Science-backed Skin Care Ingredients

Since beauty and ageing are important issues, beauty-from-within concepts are creating huge opportunities in the fast-growing skin care market. According to Global Industry Analysts, the worldwide nutricosmetics market will reach a value of $8.3 billion by 2027 [10]. Changing lifestyles and increasing consumer incomes have led to growth in the use of supplements, thus boosting sales. As consumers become better educated about the correlation between skin health and nutrition, they are also more interested in understanding how these products work together. This means that people will favour products with the greatest amount of scientific research and substantiation to support them. With natural astaxanthin, collagen peptides and hyaluronic acid, product developers can satisfy these demands and effectively target this growing market.

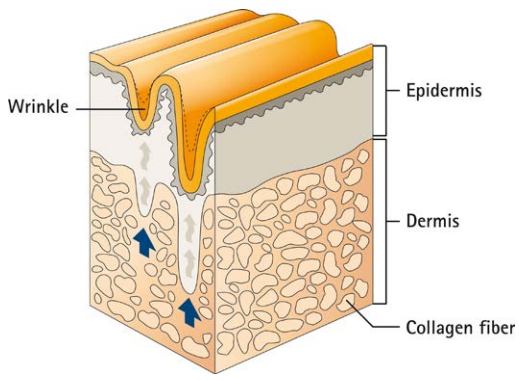

Fig. 2 Influence of collagen in the skin layers.

References

[1] *Tominaga K et al.:* Protective effects of astaxanthin on skin deterioration. J Clin Biochem Nutr. Jul 2017;61(1):33-39.
[2] *Tominaga K et al.:* Cosmetic benefits of astaxanthin on humans subjects. Acta Biochim Pol. 2012;59(1):43-7.
[3] Innova New Products Database
[4] *Proksch E, Segger D, et al.,* Skin Pharmacol. Physiol. 27, 47–55 (2014).
[5] *Proksch E, Schunck M, et al.,* Skin Pharmacol. Physiol. 27, 113–119 (2014).
[6] *Schunck M. et al.:* Dietary Supplementation with Specific Collagen Peptides Has a Body Mass Index-Dependent Beneficial Effect on Cellulite Morphology. J Med Food. 2015 Dec 1; 18(12): 1340–1348.
[7] *Kawada C et al.:* Ingested hyaluronan moisturizes dry skin. Nutr J. 2014 Jul 11;13:70. doi: 10.1186/1475-2891-13-70.
[8] *Oe M at el.:* Oral hyaluronan relieves wrinkles: a double-blinded, placebo-controlled study over a 12-week period. Clin Cosmet Investig Dermatol. 2017 Jul 18;10:267-273.
[9] *Yoon HS et al.:* Supplementing with Dietary Astaxanthin Combined with Collagen Hydrolysate Improves Facial Elasticity and Decreases Matrix Metalloproteinase-1 and -12 Expression: A Comparative Study with Placebo. J Med Food 17 (7) 2014, 810–816.
[10] https://www.strategyr.com/market-report-nutricosmetics-forecasts-global-industry-analysts-inc.asp (22.06.2020)

contact

Sandra Schulze
akp public relations
Birkenauer Talstraße 9 | 69469 Weinheim | Germany
E-mail: sandra.schulze@akp-pr.de

GELITA AG
Uferstr. 7 | 69412 Eberbach | Germany
E-mail: info@gelita.com | www.gelita.com

AstaReal AB
Forumvägen 14, Level 16 | 131 53 Nacka | Sweden
E-mail: info@astareal.se | www.astareal.se

Not Beauty is the Key to Happiness – Happiness is the Key to Beauty!

H. Döring, M. Perez-Aso, B. Martinez-Teipel, J. Bosch, A. Roca

abstract

In an environment that promotes overall wellbeing as a life philosophy, our laboratory wanted to ascertain what impact the use of a skincare product containing a new active ingredient would have on people's emotions. This novel ingredient is based on the Monk fruit (*Momordica grosvenorii*) which is cultivated in a mountain valley in Guangxi, a place known by the striking longevity and happiness of its inhabitants.

Since emotions are directly linked to the subconscious, the intention was to measure objectively the emotional impact of using this active ingredient in a cosmetic application. To do so, our laboratory used scientific rigour and Artificial Intelligence (AI) taking into account such subconscious evaluations.

But overall wellbeing is seen as integrative beauty that embraces both the emotional health and the physical aspect. Consequently, our laboratory also took a closer look at the causes for aging. While many people say it is all in the genes, Epigenetics suggests that not everything is genetically predisposed. Lifestyle has a major impact and maintaining good habits like exercising, eating plenty of fruits and vegetables, not smoking, and drinking alcohol in moderation can have very positive effects on the aging process.

Introduction: Epigenetics

Over the past decade, a growing number of studies have revealed that progressive changes to epigenetic information have a huge influence on the aging process. Several important conclusions emerge from these studies: rather than being genetically predetermined, our life span is largely epigenetically determined; lifestyle habits, diet, pollution and other environmental factors can influence our life span by changing the epigenetic information. Given the reversible nature of epigenetic mechanisms, these studies provide promising avenues for a healthy aging [1].

Epigenetics refers to heritable and reversible changes in gene expression that do not involve changes to the underlying DNA sequence; a change in phenotype without a change in genotype. This genetic control implies that genes are turned on or off through chemical modifications on the DNA (methylation or hydroxymethylation) and histones (phosphorylation, methylation, acetylation). Other gene modifiers contributing to the epigenetic regulation are the non-coding RNAs such microRNAs (miRNAs) or long non-coding RNA (lncRNAs).

MicroRNAs are a class of short, endogenous, single-stranded, noncoding RNA molecules. By binding a specific sequence of the target mRNAs, they can regulate the expression of multiple genes at the post-transcriptional level through degradation or translational inhibition of the targeted transcripts, thereby decreasing protein synthesis [2]. A single miRNA can target up to several hundred mRNAs, and one gene is targeted by many different miRNAs, therefore by reducing or increasing miRNAs levels we are able to significantly harmonize gene expression regulatory networks.

In skin, miRNAs have specific roles such as regulation of cell senescence in different environmental conditions both in keratinocytes and fibroblasts [3], miRNAs involved in the stress response in the extracellular matrix with the loss of different proteins such as collagen and elastin fibers [4] and those involved in skin diseases such as atopic dermatitis and psoriasis [5]. Therefore, miRNAs are involved in many of the pathways behind skin aging, modulating gene expression and changing the aspect of aged skin, increasing its fragility and wrinkles, impairing barrier and immune function and delaying desquamation and wound healing, leading to drier skin.

Results of the *in vitro* and *ex vivo* Efficacy Study

These assays sought to evaluate the anti-aging activity of a novel active ingredient based on the monk fruit on the skin. For this reason, *in vitro* studies are divided in two parts:

- Epigenetic miRNA signature in human cultured dermal fibroblasts isolated from aged donors.
- Histology of the components of the dermis in human skin explants *ex vivo*

Normal human dermal fibroblasts from an aged donor (66 years old) were isolated from facial skin. The active tested in these cells was a dry extract of the fruit *Momordica grosvenorii*. 250 µg/ml have been used for the *in vitro* and 100 µg/

ml for the *ex vivo* experiments, corresponding to 0.93% and 0.45% of the active ingredient respectively.
The analysis of miRNA differential expression in aged fibroblasts revealed a total of 9 novel miRNAs from 2000 differently expressed; 8 of them were down regulated whereas only one was up regulated. The reduction in the expression levels varies from 50% to 80% whereas the over expression goes to 114.6%, indicating that the active is able to limit the general miRNA upregulation seen during aging.
We were also interested in knowing what happened with miRNAs already described in the literature with validated targets on the skin. Specifically, there was also a reduction in the expression level of miR-21, miR-29a-3p, miR-29b-3p and miR-22-5p (-33%, -22%, -32% and -23% respectively). Gene validated targets of the miR-29 family are all related with extracellular matrix proteins such as collagen I, collagen V, elastin, fibrillin, whereas miR-21 target is related to ECM degradation (gene is TIMP3). Other gene targets for miR-22 are related with cellular senescence in a way that its overexpression provokes an arrest in cell growth [6]. Therefore, this miRNA downregulation suggests an increase in the synthesis of ECM components as well as in the metabolism rate in aged fibroblasts by the active ingredient.
Next, we proceeded to analyse the predicted genes and pathways likely to be involved in the regulation by the miRNAs described in the previous section. For this, all the novel miRNAs were entered in the DIANA-mirPath web server, that utilizes predicted and validated miRNA targets either with Tarbase v7.0 or microT-CDS algorithms to find out through the KEGG pathway analysis which routes are the most likely to be modulated. The biostatistical analysis revealed that the pathway most likely to be modulated by the miRNAs is the biosynthesis of glycosaminoglycans (type O - glycans) together with keratan sulfate, both being related to the proteoglycan pathway (hyaluronic acid, dermatan sulfate, keratin sulfate and heparan sulfate). This result is in agreement with the validated targets for the specific miRNAs found being modulated by the active, which means an increase in the synthesis of extracellular matrix constituents due to a reduced expression in the miRNAs levels.
To corroborate the results from the miRNA array and the analysis, we chose to study the effects of the active on the components of the extracellular matrix. For this reason, skin explants were incubated with 0.45% of the active to visualize possible changes in GAGs content as well as decorin and hyaluronic acid.
As you can see in **Fig. 1**, 0.45% active treatment (lower row) induces a very significant increase of hyaluronic acid (HABP staining) as well as an increase in decorin expression in the papillary dermis. There is also an increase in hyaluronic acid in the epidermis compared to basal conditions. Explant images were quantified showing a raise both in the presence of hyaluronic acid in the dermis (+320.7%) and decorin (+34.7%) due to the treatment with active.
In summary, the *in vitro* and *ex vivo* results show that the *Momordica grosvenorii* extract is able to rebalance the gene expression in aged fibroblasts through regulation of the miRNA expression, increasing the synthesis of different components of the extracellular matrix and fibroblast function, thus suggesting an antiaging role of our active.

Results of the *in vivo* Efficacy Study

To evaluate the efficacy of the *Momordica grosvenorii* extract physically and emotionally, two different double-blind, randomized, placebo controlled *in vivo* studies were performed with two daily applications over an 8-week period (D0, D28 and D56) and at a concentration of 2%.

Study I (physical assessments)

The evaluation consisted in instrumental analysis to assess objectively and quantitatively the anti-aging efficacy of the product on the skin by different methodologies: skin hydration with a Corneometer®, biomechanical parameters (Dual-Cutometer MPA®), skin gloss with a Glossymeter® and skin thickness, Subepidermal Low Echogenic Band (SLEB) and density by ultrasonography (Dermascan C system).
The study was performed on 44 Caucasian female volunteers aged >60 years old, phototype I to IV, with evident signs of aging. The panel was divided between volunteers applying a placebo formulation (n=23) and the active formulation (n=21) in the face and neck.

Skin Hydration
Results show that our active causes a marked increase in hydration, about 32% after 28 days of treatment and up to 67% after 56 days while the placebo increases hydration by 11% and 34% respectively.

Skin gloss
When gloss was evaluated, the increase found in volunteers treated with the active was 66% more at the end of the study compared to day 0 whereas placebo slightly rose up to 26%

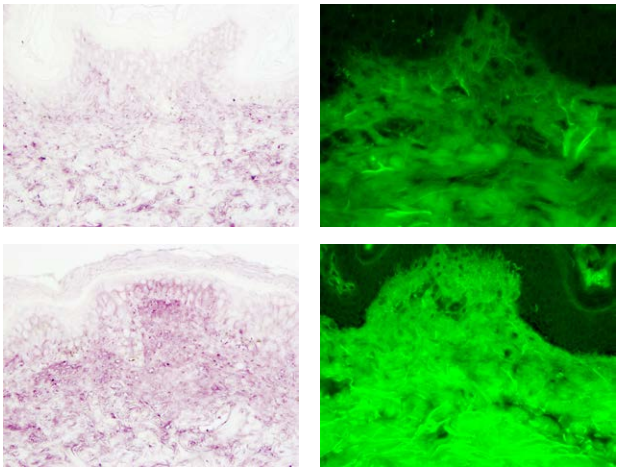

Fig. 1 Changes in HA and decorin in skin explants treated with the active. Upper row, explants in basal conditions and the lower row, explants with the active.

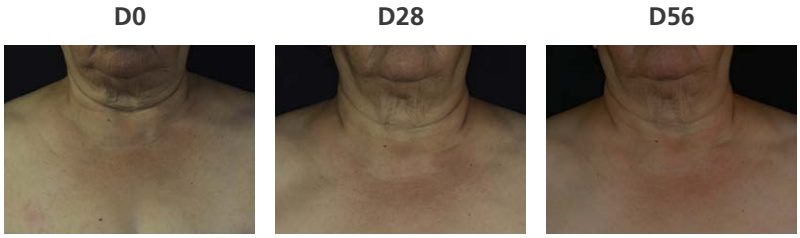

Fig. 2 Evolution of neck wrinkles reduction in volunteer 29.

Skin ultrasonography evaluation

The first parameter was the dermis density where a significant raise was observed both at D28 and D56 for the volunteers treated with the active as compared to placebo. The difference between both treatments shows an increase of 16% and 28% in the dermis density after 28 and 56 days due to the active. Moreover, the average area of the sub epidermal low echogenic band (SLEB) was also calculated and results show a decrease in the SLEB thickness both at 28 and 56 days about -8% and -15% due to the treatment with the active.
Results of both tests show that the skin structure integrity improves and gets reinforced. This is translated to a densified and plumper skin.

Skin firming effect

We also observed that the active reduces neck folds to give a smoother and rejuvenated appearance (**Fig. 2**).

Crow's feet reduction evaluation

We also observed a decrease in the number of crow's feet in volunteers treated with the active as compared to placebo; 12% reduction after 28 days and 18.5% after 56 days (**Fig. 3**).

Study II (emotional responses)

The evaluation consisted in a neurostudy based on questionnaires and videos (ImagineLab®) with the Mindlogics® technology using applied neuroscience and other cognitive sciences [7].

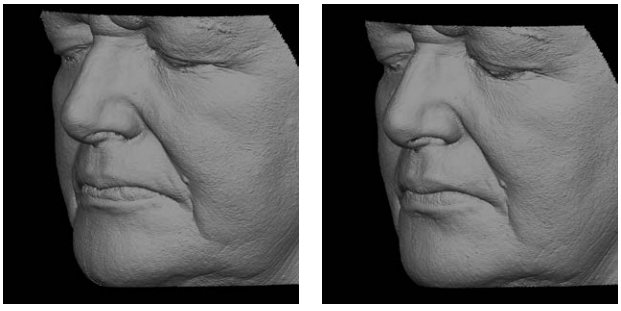

Fig. 3 Representative AEVA 3D reconstruction of the reduction in crow's feet after 56 days treatment with the active (volunteer 34).

This neurostudy was performed on 47 Caucasian female volunteers > 60 years old, phototype II to IV, with evident presence of wrinkles and all skin types (dry, combination and oily). The panel was divided between volunteers applying placebo formulation (n=23) and verum (n=24) in the face and neck.

Assessment of wellbeing (conscious vs subconscious).

Mindlogics® is the method used to understand the whys and the decision making process. It is owned and created by Kernel Business Consulting (KBC) who was our partner in this study. Mindlogics® is based on 5 mind dimensions to find out what volunteers really think, 3 subconscious (referent, enablers and experience) and 2 conscious (inhibitors and disinhibitors).
To carry out this neurostudy, different types of questions and videos were the stimuli to make the subconscious mind of the volunteers react. Besides, these questions were open-and closed-ended with different types of answers. Every panellist was in front of a computer with a camera answering the different questions and watching a video whereas ImagineLab® was registering all the facial micro expressions of the volunteers while watching and answering the questionnaire.

Emotional benefits evaluation

The direct analysis of the answers to the questionnaire expressed the conscious needs and desires of the volunteers and there was no significant difference in any of the answers between placebo and active. However, when Mindlogics® analysed all the registers to the questions and video watching, we could observe an important change. As you can see in **Fig. 4**, there is a clear difference between what volunteers think rationally and what they "really" think. These questions were about the satisfaction level after using the products and if they thought products make them happier and feel much better. We observed for the three categories that the best

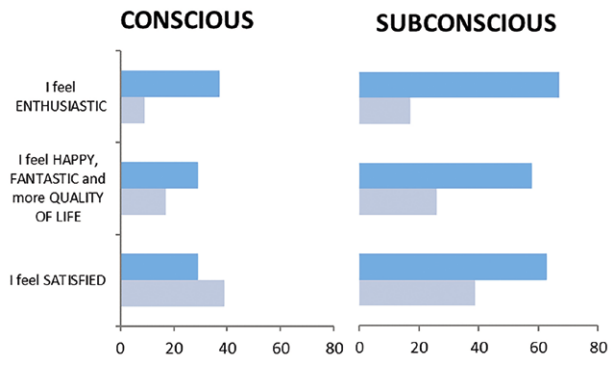

Fig. 4 Percentage of volunteers choosing the positive and top answer in both conscious and subconscious responses. Possible answers were: totally agree, somewhat agree, somewhat disagree and totally disagree for the two bottom statements whereas for enthusiasm was yes or not. Dark bars are the active and light bars are placebo.

response is always for the active at subconscious level. These results mean that volunteers treated with the *Momordica grosvenorii* extract were totally satisfied with the experience, they felt better, happier and they were excited about using our active in a subconscious manner.

Conclusion

The active ingredient based on a Monk fruit (*Momordica grosvenorii*) extract has demonstrated its efficacy acting at two different levels; having a direct antiaging effect on skin through modulation of the miRNA expression and also having an impact on the subconscious mind to make women feel great. From the *in vitro* experiments, the active rebalances the gene expression in aged dermal fibroblasts through regulation of the miRNA expression, increasing the synthesis of different components of the extracellular matrix and improving fibroblast function, thus demonstrating an antiaging role of the active.

In the clinical tests, we have also shown that the active delivers significant increased hydration and luminosity to the skin, as well as densification with a very visible reduction in the neck folds and crow's feet. Moreover, the active provides positive psychological benefits and it impacts on the subconscious of the volunteers, women feel happier, fantastic and satisfied with our active.

References

[1] *S. Pal* and *J. K. Tyler,* "Epigenetics and aging", Science Advances, vol. 2, no. 7. American Association for the Advancement of Science, 2016, doi: 10.1126/sciadv.1600584.

[2] *S. V. Thalyana* and *F. J. Slack*, "MicroRNAs and their roles in aging," Journal of Cell Science, vol. 125, no. 1. pp. 7–17, Jan. 01, 2012, doi: 10.1242/jcs.099200

[3] *M. Mancini et al.,* "MicroRNAs in human skin ageing," Ageing Res. Rev., vol. 17, pp. 9–15, Sep. 2014, doi: 10.1016/j.arr.2014.04.003

[4] *E. V. Edeleva* and *H. R. Shcherbata*, "Stress-induced ECM alteration modulates cellular microRNAs that feedback to readjust the extracellular environment and cell behavior,"Front. Genet., vol. 4, p. 305, Dec. 2013, doi: 10.3389/fgene.2013.00305.

[5] *J. E. Hawkes et al.*, "microRNAs in Psoriasis," J. Invest. Dermatol., vol. 136, no. 2, pp. 365– 371, Feb. 2016, doi: 10.1038/JID.2015.409

[6] *M. M. S, C. E. P,* and *J. García-Jiménez,* "Epigenética y envejecimiento. Claves para el desarrollo de una cosmética de nueva generación basada en la regulación de los mecanismos celulares del envejecimiento," Not. Cosmet. y Perfum., no. 348, pp. 5–13, 2016.

[7] *J. Fernández,* "Reinventar la propuesta de valor desde el subconsciente con Advanced Analytics," pp. 1–6, 2019

contact

Hagen Döring*
Miguel Perez-Aso
Blanca Martinez-Teipel
Jordi Bosch
Angela Roca

*Provital S.A.U.
Barcelona | Spain
E-mail: h.doering@weareprovital.com

Combining Science with Nature for a New Concept in Skin Fillers

J. Caverzan da Silva, P. da Luz Moreira, L. Mussi, W. Magalhães

abstract

Over time, physiological changes like the reduction of collagen and elastin production, as well as an overall decrease in skin metabolism and redox imbalance, result in significant aesthetic changes that include increased skin roughness, wrinkles, loss of elasticity and firmness, thinning of the skin, and the appearance of age spots. Additionally, bone retraction and the loss of adipose tissue (lipoatrophy) are also factors in the appearance of aging skin. Several studies have shown that the greater the facial lipoatrophy, the older it appears. To restore a more youthful appearance, dermal fillers are injected into the dermis to re-establish the volume lost to lipoatrophy. Seeking a non-invasive alternative to injectable fillers, Cellfie was developed as a plant-based ingredient that offers a precise and efficient delivery system that activates adipogenesis by stimulating adiponectin and PPARγ to ease the signs of aging. Both mediators were evaluated *in vitro* as well as the adipogenesis effect. A clinical study was performed using the Bio3D Structured-light Scanner to evaluate area, length, and depth of wrinkles on perioral, nasolabial, periorbital wrinkles and smile regions. Cellfie was found to effectively induce adipogenesis – stimulating skin filling and reducing the signs of aging.

Introduction

Adipose tissue is extremely complex. Its main function is regulating and coordinating energy homeostasis, including the coordination and control of a series of physiological processes in the body, for example, glucose metabolism, appetite, immune and inflammatory responses, angiogenesis etc. This tissue is mainly formed by cells called adipocytes, which during their development go through determination and differentiation processes until they become mature cells, characterized by a process known as adipogenesis [1].

Adipogenesis is the process by which adipose progenitor cells differentiate into cells that have the ability to store excess energy as a neutral lipid (triacylglycerol) and release it as non-esterified fatty acids and glycerol when the body's demands increase during hunger or exercise [1]. In this process, adipocytes also trigger signals to recruit more progenitors and restart the adipogenesis process [1] (**Fig. 1**). Average age of adipocytes in humans ranges from 2 to 10 years [1].As we age, lipoatrophy (the loss of adipose tissue) combines with skin physiological changes to accelerate the signs of aging. Several studies have shown that the greater the facial lipoatrophy, the older it appears [1].

There are two types of adipose tissue, which are broadly studied in mammals: white adipose tissue (WAT) and brown adipose tissue (BAT). Recently, a third type of adipose tissue has been investigated, called beige adipose tissue [2]. WAT is the predominant adipose tissue in adults, being distributed throughout the body and found mainly in the subcutaneous layer. It is responsible for storing extra energy such as triglycerides and secreting hormones and cytokines, known as adipokines, which regulate the function of various tissues [1,2]. BAT, on the other hand, is the tissue responsible for dissipating heat generating energy, and is located in specific regions in the paravertebral, supraclavicular and periadrenal regions [1,2].

Mature white adipocytes store lipids in a single large droplet that occupies 85-90% of the cytoplasm and pushes the nucleus and a thin layer of cytosol to the cell circumference [3]. These adipocytes are responsible for storing 80% of body fat and for the subcutaneous deposits [1]. Brown adipocytes contain several small droplets of lipids inside and a large number of mitochondria that can convert chemical energy into

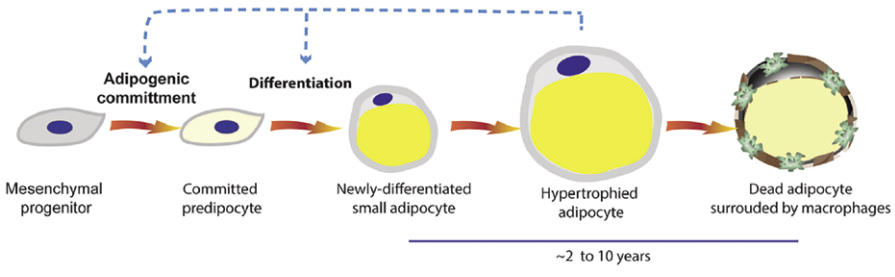

Fig. 1 Life cycle of adipocytes – determination and differentiation throughout adipogenesis. Scheme from *Lee MJ* [1].

heat [2]. The beige adipocyte is similar, morphologically, to brown and is also characterized by its ability to expend energy through thermogenesis [4].

Brown adipocytes appear in anatomically distinct regions of white adipocytes (mainly interscapular in babies and paraspinal and supraclavicular in adults). The beige ones, on the other hand, are found together with WAT (mainly in subcutaneous deposits) [4].Despite differences in location and function between white, brown and beige adipocytes, the three cell types share many differentiating features and are regulated by different factors during adipogenesis.

Adipogenesis is considered a dynamic process, which can be divided into two phases: the first one is called the determination or commitment phase, and the second one is known as the adipocyte differentiation phase [1,2]. In the first phase, multipotent cells (stem progenitor cells) are differentiated into predipocytes. In the second phase, predipocytes proliferate (clonal expansion), which then generate mature adipocytes (**Fig. 1**). These cellular events are controlled by different molecular mediators, which may be specific targets to induce or inhibit adipogenesis [4].

When multipotent stem cells generate predipocytes, Wnt signaling pathways are involved – these are a family of glycoproteins secreted by paracrine and autocrine mechanisms. Increased Wnt pathway expression through Wnt1 gene suppresses adipogenesis, while its inhibition promotes the induction of key transcription factors in this process, which are C/EBPα and PPARγ [4]. In adipocyte differentiation, the transcription factors C/EBPβ and C/EBPδ are activated. These, in turn, activate the transcription factors C/EBPα and PPARγ, which induce the transcription of several genes related to this cell type and the storage of lipids, such as the enzymes fatty acid synthase, lipoprotein lipase, lipin 1, acyl-CoA diacylglycerol acyltransferase 1 (DGAT1), in addition to lipid-carrying proteins such as the FABP4 protein and hormones, e.g. adiponectin, which is related to the proliferation and differentiation of adipocytes, in addition to increased lipid content [2,5].

Peroxisome proliferator-activated receptors (PPARs) are a group of three nuclear receptor isoforms, PPARγ, PPARα and PPARδ, encoded by different genes. PPARs are binder-regulated transcription factors that control gene expression by binding to specific response elements (PPREs) [6].

Under the action of agonists (activators), PPAR conformation is altered and stabilized, creating a binding site, with subsequent recruitment of transcriptional coactivators, thus resulting in increased gene transcription [5,6]. Endogenous PPAR binders are not yet fully known; however, various lipid metabolites including polyunsaturated fatty acids and eicosanoids have been identified as binders of these receptors, which act therefore as lipid sensors and regulators of lipid metabolism [6,7].

Showing evidence of its importance in lipid metabolism, *in vivo* studies have shown that PPARγ-specific deletion of adipocytes blocks high-fat diet-induced obesity by suppressing

adipocyte differentiation and inhibiting the increase in lipid content [8].

Adiponectin (also known as ACRP30, apM1, adipoQ and GBP28) is a hormone produced exclusively by adipose tissue cells. This protein is dramatically regulated during adipogenesis and remains one of the most specific adipocyte gene products identified so far [9]. In addition, just like other adipokines, it is highly expressed and secreted by mature adipocytes, making it an excellent biomarker in the process of increased adipose tissue [8].

Adiponectin is known to be regulated by various transcription factors such as PPARγ, (C/EBP)α and sterol regulatory element binding protein 1c (SREBP1c) [7,9]. Studies reveal that PPARγ binders, endogenous or exogenous, can induce the expression of the adiponectin gene [10], therefore, both are key biomarkers of adipogenesis.

Adiponectin acts in an autocrine and paracrine manner in adipose tissue and in an endocrine manner in distal tissues. In experiments involving adipocytes overexpressing adiponectin, cell differentiation was accelerated [9,11]. In addition to promoting cell proliferation and differentiation of predipocytes into adipocytes, adiponectin may increase the lipid content and insulin responsiveness of the glucose transport system in these cells [9].

The relationship between increasing fat tissue to reduce wrinkles is already well understood in the dermatology. In fact, it is comparable to the effects of injection procedures (fillers), such as hyaluronic acid, a common facial rejuvenation procedure [12]. In fact, the global market for facial fillers reached $3.5 billion USD in 2018, and an annual growth of approximately 8% is expected through 2026 [13]. North America is responsible for 45% of this market, followed by Europe (Germany, UK, & Italy) and Asia-Pacific regions, which will have the fastest market growth between 2019 and 2026 due to a rapidly aging population, rising per capita disposable income, and an escalating demand for facial beauty products and procedures [14].

Dermal fillers are gel-like substances, which, when injected into the dermis, re-establish the volume lost to lipoatrophy and restore a more youthful appearance. Hyaluronic acid represents 77% of this market [13]. However, it often results in an unnatural appearance due to the injection of fillers into the dermis, an extremely fibrous skin layer composed of collagen and elastin, that is naturally resistant to this type of procedure.

In addition, even though it is one of the most cost-effective anti-aging treatment procedures and eliminates downtime or surgery, dermal fillers are still considered an invasive procedure and may require the possible use of a local anesthetic that needs to be performed by specially trained clinicians.

A non-invasive alternative, just as effective as injections, can be achieved by developing products capable of reaching adipocytes and stimulating adipogenesis. The activation and proliferation of adipose tissue cells, as well as the expansion of mature adipocytes, plays a crucial role in increasing the volume of tissues, such as the skin [15]. Therefore, regular maintenance of adipose tissue through the application of pro-adipogenesis substances can prevent or even reduce the signs of aging skin. This provides a viable alternative to the use of needles.

While searching for a non-invasive alternative to dermal facial fillers, a plant-based ingredient with a precise and efficient delivery system for activating adipogenesis by stimulating adiponectin in order to ease the signs of aging was developed. Cellfie consists of the synergistic association of the extract of *Thymus vulgaris* (thyme) with phosphatides, triglycerides and lecithin fatty acids for a liposome delivery system.

Thyme has been used in Chinese and Indian traditional medicine for respiratory diseases (cough, bronchitis and asthma), toothache, urinary tract infection, dyspepsia and skin problems such as oily skin, acne, dermatitis and insect bites [16,17]. In fact, thyme contains numerous phenolic compounds, especially thymol, carvacrol, trans cinnamic acid, rutin and chlorogenic acid [18], which are related to its biological effect. Phenolic compounds such as derivatives of caffeic and rosmarinic acid [19] were described in wild thyme and may be related to its ability on regulating agent of adipocyte differentiation.

Actually, rutin had shown to increase lipid accumulation and the expression of transcription factors, such as PPARγ, (C/EBP)α and adipocyte fatty acid binding protein (FABP4) [20]. It also upregulated adiponectin expression and secretion during adipocyte differentiation [21]. In addition, rutin may be related to thyme's ability to induce and control adipogenesis. Chlorogenic and trans-cinnamic acids also promote adipocyte differentiation, acting as a potential PPARγ agonist and stimulating the secretion of adiponectin, respectively [22,23].

However, adipose tissue localization imposes a challenge for the pro-adipogenesis strategy for filling the wrinkles. Thus, a lecithin liposome system was used in the development of Cellfie's bioactive extract. Lecithin plays different roles in the pharmaceutical, cosmetics and food industries as emulsifier, viscosity modifier, stabilizer, solubilizer and penetration enhancer [24] and it was therefore, the best delivery system option for the challenge.

Materials and Methods

In vitro evaluation of adipogenesis

In order to detect adipogenesis, predipocytes differentiated from 3T3-L1 fibroblasts were seeded in a 24-well plate and grown in a humid atmosphere at 37°C in the presence of 5% CO_2, using a specific culture medium until complete differentiation. Then, the cell cultures were incubated with non-cytotoxic concentrations of the thyme extract in a delivery system for 7 days.

After incubation period, cells were stained with the AdipoRed™ dye, which has the property of binding to intracellular lipids, for 15 minutes. Immediately, the images were recorded by fluorescence optical microscopy (DM1000, Leica) to qualitatively assess the increase in adipogenesis compared to the control.

Clinical study

Clinical evaluation was performed on women aged 40 to 60 years, in which 2 groups of 20 volunteers each underwent

topical treatment on the face and neck twice a day for 60 days, with a placebo gel formulation or a gel formulation with 2% Cellfie. The test was completed by 18 volunteers in the placebo group and 19 volunteers in the group that used the Cellfie formulation.

The areas evaluated for efficacy in reducing wrinkles are as follows: perioral wrinkles, crow's feet (periorbital wrinkles), smile and nasolabial lines (**Fig. 2**).

In order to assess wrinkles, different 2D images were obtained from the Bio3D Structured-light Scanner before the start of treatment (day 0), after 30 days of treatment (day 30) and after 60 days of treatment (day 60). Images were processed to generate 3D reconstructions in order to measure the depth of the wrinkles in each volunteer for every time-point.

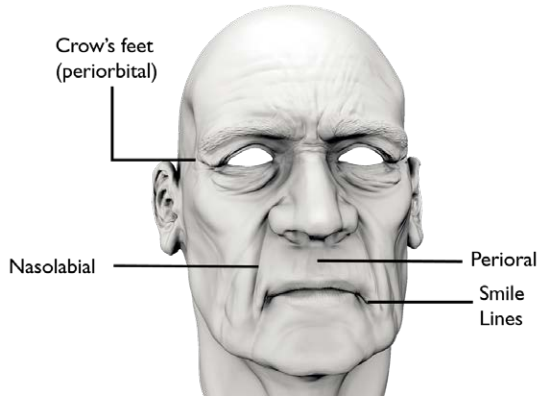

Fig. 2 Scheme showing the areas evaluated for wrinkles: perioral wrinkles, nasolabial lines, crow's feet (periorbital wrinkles) and smile lines.

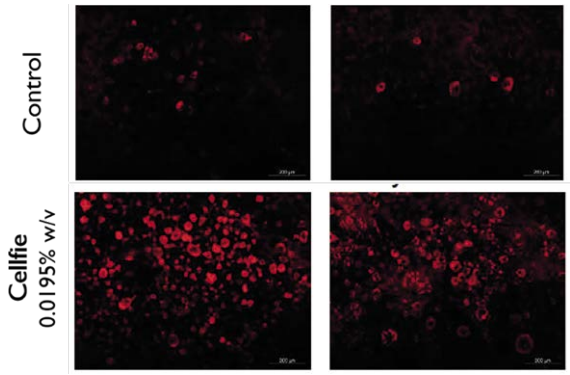

Fig. 3 Assessment on the induction of adipogenesis in the culture of predipocytes. Microscopy images at 40x magnification.

Results

Thyme extract in a delivery system was able to significantly induce adipogenesis compared to the control (**Fig. 3**) thus demonstrating significant efficacy in the accumulation of fat in predipocytes. Evaluation of PPARγ and adiponectin was also performed and it showed that adipogenesis happens due to the stimulus of both key markers (data not shown).

In clinical studies, Cellfie significantly reduced the depth of perioral wrinkles by 4.5%, after 60 days (**Fig. 4**) compared to D0, reaching up to 9% improvement in some cases. In 30 days, Cellfie has already shown a significant reduction of 2% in the depth of perioral wrinkles.

In the case of periorbital wrinkles, Cellfie significantly reduced by 3% and 4% the depth of this type of wrinkle after 30 and 60 days compared to D0, respectively. In 60 days, Cellfie obtained up to 9,5% improvement on this region (**Fig. 5**)

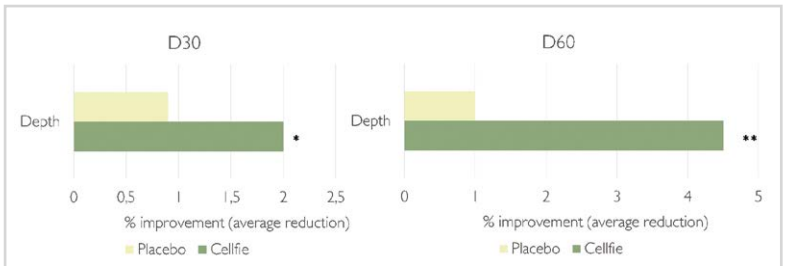

Fig. 4 Effect in the depth of perioral wrinkles. *p<0.01, **p<0.0001 compared to D0 (Paired Student's t-test).

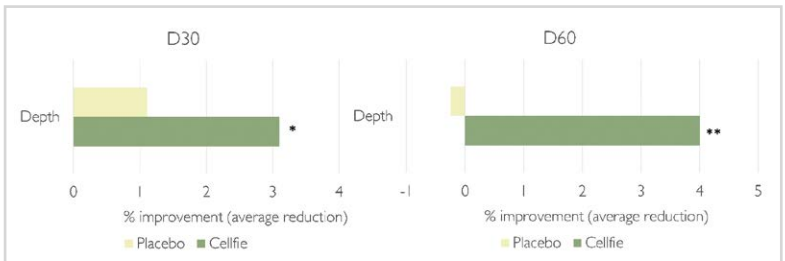

Fig. 5 Effect in the depth of periorbital wrinkles (crow's feet). *p<0.0001, **p<0.001 compared to D0 (Paired Student's t-test).

Cellfie also showed significant results after 60 days, reducing the depth of smile lines by 4% respectively (**Fig. 6**) but achieving up to 29% improvement in the best cases.

Finally, during the assessment of nasolabial lines, although there was no significant improvement in the depth of this wrinkle considering the average results (**Fig. 7**), depth was improved in some cases by up to 8% after 60 days.

The results of filling in the wrinkles in the four evaluated regions can be easily seen in the photographic documentation obtained during the study (**Fig. 8**), thus confirming the exceptional effectiveness of the product.

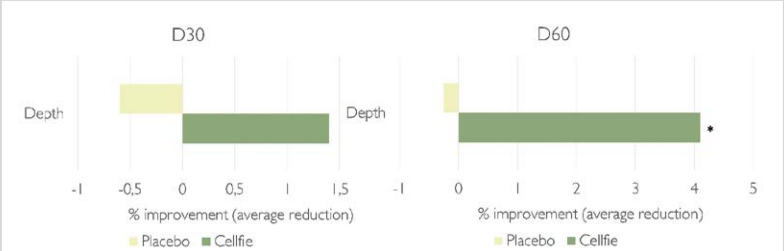

Fig. 6 Effect in the depth of smile lines. *p<0.05, compared to D0 (Paired Student's t-test).

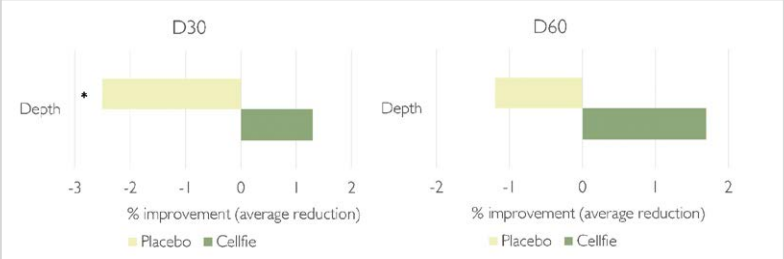

Fig. 7 Effect in the depth of nasolabial lines. *p<0.05, compared to D0 (Paired Student's t-test).

Discussion

Adipocytes from different facial fat compartments exhibit distinct morphological properties. Nasolabial compartment adipocytes are significantly larger than the adipocytes from the deep medial cheek compartment [24]. These differences are definitely responsible for significant change of the local adipose volume and thus directly influence the skin appearance in specific regions. Larger but not smaller adipocytes display inhibition of synthetic activity in adjacent fibroblasts, influencing the production of collagen, elastin and even cellular turnover [24]. Thus, areas with larger adipocytes such as the nasolabial will produce facial areas with more evident wrinkles that are a result of not only lipoatrophy but also more palpable physiological changes. This might explain the differences in the performance obtained

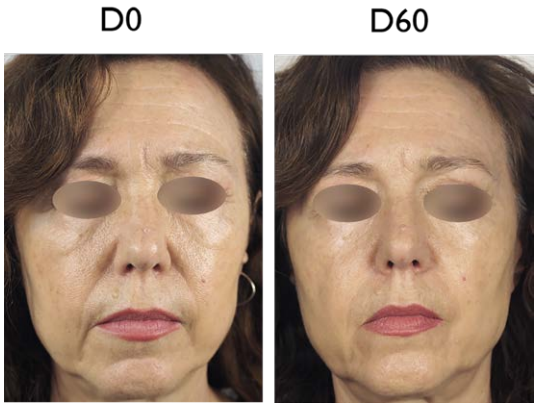

Fig. 8 Effect on perioral, periorbital, smile line and nasolabial wrinkles after 60 days of treatment.

with traditional anti-aging treatments on different regions of the face.

While in all evaluated areas Cellfie obtained a positive improvement on the depth of the wrinkles, nasolabial fold results were not significant (only after 60 days) when evaluating the average results. However, what is remarkable is the ingredient's ability to reverse the deepening of nasogenian fold caused by the placebo, and to overcome the improvement levels obtained in 30 days. Moreover, individual analysis of the volunteers had shown significant improvements of up to 8% after 60 days. Combining a pro-adipogenesis ingredient such as Cellfie with an ingredient that would stimulate fibroblasts and extracellular matrix production would certainly improve results on this region and should be considered when formulating.

This new plant-based facial filler is a major advancement in naturally reducing the look of aging skin caused by lipoatrophy, a concept related to the loss of adipose tissue in the face. As a non-invasive alternative to injections and grafting, it showed to be just as effective in reducing the appearance of facial lines, creases and wrinkles during clinical trials. Adipogenesis, the activation and proliferation of adipose tissue cells, as well as the expansion of mature adipocytes, plays a crucial role in increasing the volume of skin tissue. A regular application program of a pro-adipogenesis product will effectively maintain the adipose tissues and can prevent, or even remedy, the signs of facial aging.

Considering the ever-increasing concerns of consumers with safety and discomfort associated with invasive skin treatments, there is a significant market opportunity for natural products capable of generating similar results. Cellfie is a precise and efficient liposome delivery system that could stimulate the main biological markers responsible for the regulation of adipogenesis thus restoring cellular memory and resulting in a remarkable, natural rejuvenation of the skin's appearance.

Conclusions

An efficient, accurate, non-invasive system for delivering *Thymus vulgaris* (thyme) extract was developed, yielding a positive influence on adipocytes for skin and wrinkle lipo-filling stimulation, providing a remarkable, natural-looking result, and improving structural aging caused by lipoatrophy or loss of adipose tissue.

References

[1] *Lee MJ.* Hormonal Regulation of Adipogenesis. Compr Physiol. 2017: 7(4):1151-1195.

[2] *Chen MH, Tong Q.* An update on the regulation of adipogenesis. Drug Discovery Today: Disease Mechanisms, 2013: 10(1-2): e15-e19.

[3] *Fonseca-Alaniz MH, Takada J, Alonso-Vale MI, Lima FB.* The adipose tissue as a regulatory center of the metabolism. Arq Bras Endocrinol Metabol. 2006: 50(2):216-29.

[4] *Ghaben AL, Scherer PE.* Adipogenesis and metabolic health. Nat Rev Mol Cell Biol. 2019: 20(4):242-258.

[5] *Mota de Sá P, Richard AJ, Hang H, Stephens JM.* Transcriptional Regulation of Adipogenesis. Compr Physiol. 2017: 7(2):635-674.

[6] Berger J, Moller DE. The mechanisms of action of PPARs. Annu Rev Med. 2002: 53:409-35.

[7] *Moseti D, Regassa A, Kim WK.* Molecular Regulation of Adipogenesis and Potential Anti-Adipogenic Bioactive Molecules. Int J Mol Sci. 2016: 17(1): pii: E124.

[8] *Chang E, Kim CY.* Natural Products and Obesity: A Focus on the Regulation of Mitotic Clonal Expansion during Adipogenesis. Molecules. 2019: 24(6). pii: E1157.

[9] *Fang H, Judd RL.* Adiponectin Regulation and Function. Compr Physiol. 2018 Jun 18;8(3):1031-1063.

[10] *Iwaki M, Matsuda M, Maeda N, Funahashi T, Matsuzawa Y, Makishima M, Shimomura I.* Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. Diabetes. 2003: 52(7):1655-63.

[11] *Balsan GA, Vieira JL, Oliveira AM, Portal VL.* Relationship between adiponectin, obesity and insulin resistance. Rev Assoc Med Bras 2015: 61(1):72-80.

[12] *Kruglikov IL, Wollina U.* Soft tissue fillers as non-specific modulators of adipogenesis: change of the paradigm? Exp Dermatol. 2015: 24(12):912-5.

[13] Fortune Business Insights. Dermal Fillers Market 2019. Available at https://www.fortunebusinessinsights.com/industry-reports/dermal-fillers-market-100939. Accessed on Jan, 29th, 2020.

[14] Market Research Future. Available at https://www.marketresearchfuture.com/reports/dermal-fillers-market-3893. Accessed on Mar 11th, 2020.

[15] *Kruglikov IL, Scherer PE.* Skin aging: are adipocytes the next target? Aging (Albany NY). 2016: 8(7):1457-69.

[16] *Hosseinzadeh S, Jafarikukhdan A, Hosseini A, Armand R.* The Application of Medicinal Plants in Traditional and Modern Medicine: A Review of *Thymus vulgaris*. International Journal of Clinical Medicine 2015: 6: 635-642.

[17] *Reddy V P, Kandisa RV, Varsha PV, Satyam S.* Review on *Thymus vulgaris* Traditional Uses and Pharmacological Properties. Med Aromat Plants 2014: 3(3): 164- 166.

[18] *Rtibi K, Selmia S, Wannesa D, Jridib M, Marzoukia L, Sebaia H.* The potential of *Thymus vulgaris* aqueous extract to protect against delayed gastric emptying and colonic constipation in rats. RSC Adv. 2019: 9: 20593-20602.

[19] *Komaki A, Hoseini F, Shahidi S, Baharlouei N.* Study of the effect of extract of *Thymus vulgaris* on anxiety in male rats. J Tradit Complement Med. 2015: 6(3):257-61.

[20] *Naowaboot J, Chung CH, Choi R.* Rutin Stimulates Adipocyte Differentiation and Adiponectin Secretion in 3T3-L1 Adipocytes. J Med Assoc Thai. 2015: 98 Suppl 3:S1-6.

[21] *Peng SG, Pang YL, Zhu Q, Kang JH, Liu MX, Wang Z.* Chlorogenic Acid Functions as a Novel Agonist of PPARγ2 during the Differentiation of Mouse 3T3-L1 Predipocytes. Biomed Res Int. 2018: 8594767.

[22] *Kopp C, Singh SP, Regenhard P, Müller U, Sauerwein H, Mielenz M.* Trans-cinnamic acid increases adiponectin and the phosphorylation of AMP-activated protein kinase through G-protein-coupled receptor signaling in 3T3-L1 adipocytes. Int J Mol Sci. 2014: 15(2):2906-15.

[23] *Peng SG, Pang YL, Zhu Q, Kang JH, Liu MX, Wang Z.* Chlorogenic Acid Functions as a Novel Agonist of PPARγ2 during the Differentiation of Mouse 3T3-L1 Predipocytes. Biomed Res Int. 2018: 8594767.

[24] *Wollina U, Wetzker R, Abdel-Naser MB, Kruglikov IL.* Role of adipose tissue in facial aging. Clin Interv Aging. 2017 Dec 6;12:2069-2076.

contact

Jeanifer Caverzan da Silva | MSc, Skin Care R&D Leader, Chemyunion
Patricia da Luz Moreira | PhD, Skin Care Product Manager, Chemyunion
Lilian Mussi | MSc, Innovation Manager, Chemyunion
Wagner Magalhães | MSc, R&D Director, Chemyunion

Corresponding author:
E-Mail: patricia.moreira@chemyunion.com

Full Spectrum Hyaluronans: Evidence of Effectiveness by Fine-tuning Development of Cell Physiology

A. Maltagliati, S. Zanzottera

abstract

The present paper aims at demonstrating the overcoming concept of standard Sodium Hyaluronate (SH) and its acidic form (HA).

The basic notion about Sodium Hyaluronate is that different molecular weights can stimulate different singular effects on cells, leading to opposite physiological performances.

2.0 Full Spectrum Hyaluronans provide an "ecological" beauty routine for skin because they reproduce human physiological requirements by focusing on a wide spectrum, which mediates a specific cellular answer through the activation of different cell receptors and the fine-tuned effect of skin regeneration processes in a physiological-like stimulation.

Introduction: Hyaloronan Characteristics

Hyaluronan (HA) is a polymer, part of glycosaminoglycans (GAG) along with heparan sulfate or chondroitin sulfate. It is a linear flexible high molecular mass polysaccharide that consists in the repetition of a disaccharide monomer constituted of D-glucuronic acid and N-acetyl-D-glucosamine. The repetition of the basal unit (usually from 2 000 to 25 000 times) leads to a component with a molecular weight of at least 1 000 kDa that can extend its length from 2 to 25 µm. This is a very important component of our skin. Present in the epidermis, it is mainly contained in the dermis, and both compartments account for around 50% of the total body HA. The rest can be found in particular in extra-cellular matrix of connective tissues. It is estimated that a 70-kg individual presents about 15g of HA [1] in the whole body, of which a third is renewed every day. Thus, the half-life of HA found in our skin is about 1 day.

The molecular properties of HA confer distinct functions on it. The main one is its contribution to tissue homeostasis as a result of its biophysical properties. Being the only non-sulfated GAG, and thanks to its important size, HA forms a gel matrix capable of binding great amounts of water. It forms a stiffened and expanded random coil with strong hydrogen bonds between adjacent sugar units, like a spongy grid of support upon which the superficial epidermis relies. HA can also interact with link-proteins and hyaladherins (non-covalently bound with a number of HA-binding proteins), adding to its importance in the structural integrity of extra- and pericellular matrices. Last, but not least, HA also interacts with cell surface receptors such as RHAMM or CD44.

From homeostasis state, a degradation in low molecular weight hyaluronan (LMW HA) reduces the anti-mitogenic effect of CD44 due to the active quiescence of HMW HA. This simultaneously increases the pro-mitogenic effect of LMW. The combination in the regulation of these two contradictory effects provides a rheostatic control, much more sensitive and efficient than a simple "on/off" switch for mitogen control. This regulatory mechanism probably allows the cell to react in a more sensitive, fine-tuned manner, therefore opening a much more subtle range of physiological responses.

Varying forms of a molecule characterized with the same INCI name could lead to opposite physiological performances. For instance, a LMW HA is known to stimulate inflammatory mediators while a high molecular weight hyaluronan (HMW HA) tends

to the opposite physiological reaction. In such cases, it therefore becomes very important not only to characterize the chemical nature of the active ingredient, but also its real efficacy.
Skins are as different as molecular weights are. They react in specific, varied ways, but are all based on the same pool of native ingredients, among which are the dominant hyaluronans. Just like our skin, the physiology of this polymer varies with its environment, essentially due to its molecular weight. Recent studies have shown the stimulative influence of a LMW HA in stress conditions as opposed to the calming influence of a HMW in homeostatic phase [1,2]. The capacity to deliver a specific spectrum of molecular weight instead of a common single peak consequently makes biological sense when considering the possible synergies between the different molecules.

CD44 Receptor Characteristics

CD44 is a transmembrane glycoprotein encoded by a single gene and expressed as several isoforms, due to intensive alternative RNA splicing (insertion of up to 11 additional exons into a site within the membrane-proximal portion of the 248 amino-acids extracellular domain), and differential post-translational modifications such as glycosylation or the attachment of glycosaminoglycans. However, the HA binding site is present on all isoforms, and our focus will be on the predominant standard form.
Many studies have been led on the interactions between receptor CD44 and its ligand, as it is a convergence point for many mechanisms in the physiology of important cells, including skin cells such as keratinocytes or fibroblasts. The capacity of HA to directly modify cell behavior had already been understood since 1980, when two demonstrations showed specific binding with intact cells [2,3] and the enhancement of cell motility in two-dimensional cultures [4]. Later studies showed that the ability of HA to activate intracellular signaling cascades required interactions with hyaladherins, but was additionally modified by the amount and size of HA present in the environment of the cells [2].
This trend of perception has been expanding lately, admitting that it becomes increasingly evident that mesenchymal cells (such as fibroblasts) are provided with the capacity to sense changes in their local environment (a volume in which the cells are evolving), especially when homeostasis is compromised. In this case, cell surface receptors are the first exposed to detect even sensible changes in the local tissue environment, and can lead to the first cellular answers to re-establish homeostasis or tissue integrity. In this case, Hyaluronan signaling involving CD44 is coupled with downstream pathways that can consequently be greatly influenced by the size of external hyaluronan constituting the cell background.

CD44/Hyaluronan Interactions

The smallest hyaluronan fragment binding to a CD44 is a hexasaccharide [5], and latest studies evaluated *in vitro* that HA with molecular weight up to 10kDa could be bound reversibly to its specific receptor, whereas the binding capacity of HA with a molecular weight superior to 30kDa could be estimated as irreversible [6] as it probably is bound to many CD44 at the same time. However, this binding affinity (regulating ligand/receptor interaction) is itself regulated by the expression of CD44. In fact, CD44 functionality seems to depend on the cellular context (in activation/deactivation mechanism). However, in the case of cells such as fibroblasts, they are constitutively active, always on the "ON" position, but appear to leave much more subtlety in the possibilities of influencing fibroblast physiology.

New scientific opinions describe the difference of physiochemical properties of LMW HA versus HMW HA and the consequent adverse cell reaction due to a distinct interaction with CD44. When skin integrity is compromised (wound, oxidation), an ensemble of reactions tends to cut HMW HA into fragments, considered as "danger" signals [7]. Invading pathogen bacteria can also cut their way through with hyaluronidases, therefore adding to the presence of LMW HA. These fragments revealed multiple pro-inflammatory potentials, which were not observed for HMW HA. Moreover, HMW HA can block the pro-inflammatory effects of LMW HA, opposing here an active way to maintain homeostasis (a "sound of silence"), when many scientists thought HMW HA was inert. The opposing cell cycle effects of HMW and LMW result from differential regulation of CD44-regulated signaling pathways to cyclin D1 [8,9], and emphasize how a subtle change of composition in the cell micro-environment (extra cellular matrix) can lead to adverse reactions, LMW HA reacting in a completely opposite manner to HMW HA [7,8].

A very interesting hypothesis on mechanism of action suggests that HMW would inhibit cell proliferation by antagonizing mitogen-dependent cyclin D1 expression by stimulating merlin dephosphorylation, CD44-merlin association and CD44-ERM protein dissociation. This would lead to a growth-inhibitory state where HMW actively installs a state of quiescence. On the opposite, LMW binding to CD44 would lead to hyper-phosphorylation of merlin and CD44-ERM protein association [10]. This would eliminate the inhibitory effect of CD44-merlin couple on Rac activation (a signaling pathway identified in many cell reactions). The CD44-merlin couple would therefore act like a molecular switch.

Full Spectrum Technology

The industrial production of cosmetic hyaluronan has been going through many changes in the past decades, from the animal extraction of rooster combs to the supervised biofermentation by bacteria such as streptococcus or bacillus species. The discovery of this polymer has not given immediate access to its synthesis, and for years, the only way to obtain it had been from animal origin, with uneven results in regards to the molecular weight and its statistical repartition around a medium peak value. On the contrary, the control of biotechnological production parameters allows for obtaining a specific quite narrow range of molecular weight, centered on medium values as low as 20 kDa, and up to 3 000 kDa and even more. Today's technologies are able to reach even smaller components (oligomer HA) or bigger ones (cross-linked HA). Therefore, what was not considered as important just a few years ago became a crucial parameter in the recent identification of a sodium hyaluronate: its molecular weight. And this, even if the analytical methods still not consent to a complete and precise identification of all molecule lengths potentially present in a same batch.

Full Spectrum hyaluronans are biotechnological ingredients that reproduce a specific spectrum of molecular weight, with a targeted focus on skins at a particular physiological step (homeostasis or stress). Tested stressed skins react in a more adapted manner, thanks to an evolutionary interaction between matrix hyaluronans and their cell receptor CD44. This adaptive answer lends the cell a capacity to "sense" its microenvironment and respond in return (by signal transduction or by cytoskeletal dynamics regulation). The benefit for final consumers? A better effect linked to a more specific cellular answer.

PrincipHYAL® products have been developed on the simple observation that the skin does not present just one very precise length of hyaluronan, but rather a coexistence of molecular weights, leading to specific biological mechanisms. The attention therefore became more focused on the ingredient activity, more than its molecular weight. As a matter of fact, it has been observed that a cell submitted to LMW HA can react in a completely opposite manner to a HMW HA. Studies on mesenchymal cells showed that an inflammatory reaction induced by LMW HA could be opposed to an anti-inflammatory reaction linked to the presence of HMW HA.

As per different *in vitro* cell models, a subtle modification in the molecular weight spectrum of environmental (matrix) hyaluronans (HA) could lead to modifications in the physiology of the cells exposed. We proceeded to the production of a specific quality of hyaluronan maintaining the same purity levels, but focusing less on a narrow peak, and more on a spectrum of peaks, reproducing the ones observed with the skin models. The purity of the ingredient was therefore not degraded, but the peaks have been enlarged and multiplied.

Subtler Ingredients, Fine-tuning Skin Physiology

ROELMI HPC confirmed these observations with the development of distinct hyaluronan ingredients belonging to PrincipHYAL® Line:

- PRINCIPHYAL® SIGNAL-10: quicker permeation than classic form of hyaluronans for an immediate effect of moisturization and elasticity. Very useful for the concept of TURBO cosmetic;
- PRINCIPHYAL® AURORA: a proved regenerating and wound-healing effect for a completely new skin;

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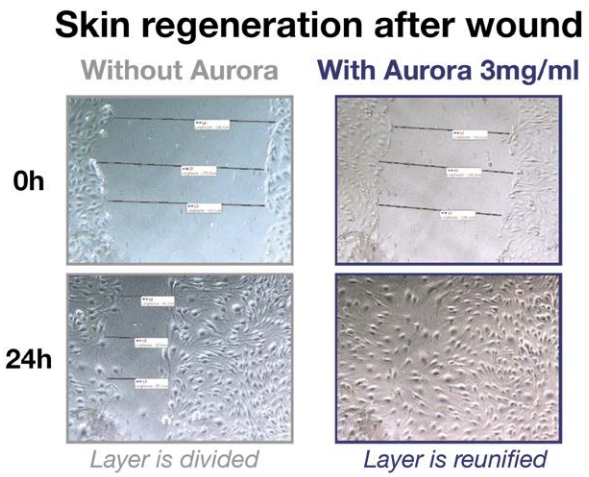

Fig. 1 *in vitro* study: Qualitative and quantitative evaluation of wound closing efficacy on an artificial wound model, by causing an artificial wound all along a confluent cell monolayer of mouse endothelial cerebral cells. Evaluation at 24 hours after the incubation of the cells with different concentration (0,03; 0,3 and 3mg/ml). The measured endpoint (wound width) takes into consideration the effect on determining cell proliferation and migration of the test system cells all along the artificial wound.

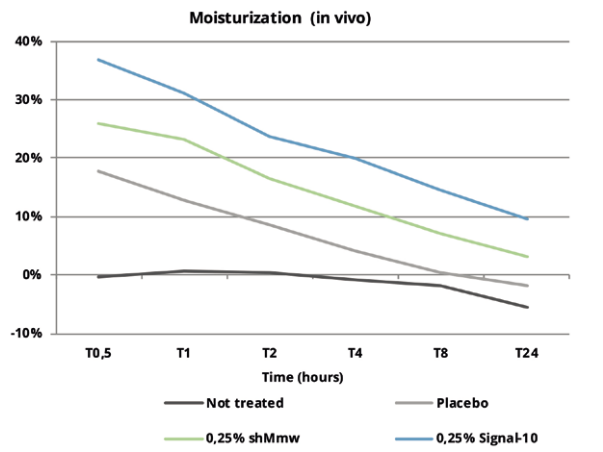

Fig. 2 *in vivo* study: on 10 women, age 18 to 65. Material: Corneometer®. Skin moisturization is measured at T0.5, T1, T2, T4, T8 and T24 after single cream application. Moisturization is measured on non-treated area, placebo cream area, 0,25% medium mw sodium hyaluronate cream area, and 0,25% SIGNAL-10 cream area.

- PRINCIPHYAL® DIFFERENCE: a wide spectrum of molecular weight studied to get long-term anti-aging on mature skins;
- PRINCIPHYAL® CUBE3: a wide spectrum of molecular weight studied to get short-term lifting effect (just 30min), with a proved efficacy of carrier for both lipo- and water-soluble ingredients.

These actives, based on determined spectrums of molecular weight (instead of just one peak), are obtained through the control of degradation parameters during the biofermentation production process. Selected from a screening of numerous *in vivo* data, they target a precise cosmetic performance, with surprisingly higher results.

PrincipHYAL® line is made of ingredients with selected ranges of different molecular weights, targeting the activation of particular cell answers. PrincipHYAL® line gives the chance of a multi-shade signal, more efficient than a simple "on/off" stimulus. The signals arriving to cells are multiple and contradictory, but their integration allows fine-tune physiological answers. A multi-shade signal allows a fine-tuned answer, more efficient than an "all or nothing" signal.

Evidence of Efficacy

Different activities performed by PrincipHYAL® ingredients have been evaluated by *in vitro* & *in vivo* clinical tests done in an external and independent laboratory with Certified Quality Management System UNI EN ISO 9001:2008 and recognized as Testing Facility operating in GLP (Good Laboratory Practices – Dir 2004/9/EC and 2004/10/EC) by the Italian Ministry of Health.

Here a summary of obtained results with several benefits:

- WOUND HEALING: PrincipHYAL® AURORA clearly marks a stimulating effect on wound healing of endothelial cells in culture after 24h. Wound widths are compared to untreated negative control (**Fig. 1**).
- MOISTURIZATION: 0.25% PrincipHYAL® Signal-10 statistically induces a constant improvement of moisturization versus medium m.w. hyaluronate (+10.9% after 30 min) (**Fig. 2**).
- SHORT TERM LIFTING EFFECT: 0.5% PrincipHYAL® Cube3 significantly diminishes wrinkles number versus placebo (down to 6.81% and 4.53% in 30 and 120 minutes) (**Fig. 3**).
- LONG-TERM LIFTING EFFECT: 0.5% PrincipHYAL® Difference formulated at diminishes wrinkle volume versus placebo (-4.4% and -12.0% after 15 and 60 days respectively) (**Fig. 4**).

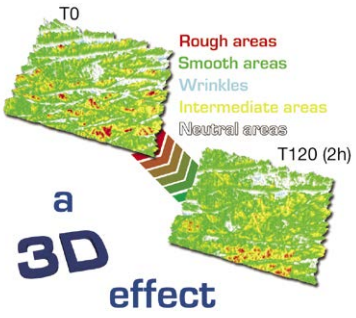

Fig. 3 3D visual representation based on integral mathematics manipulation: on 10 women aged 30 to 50. Material: Visioscan. Image analysis of parameters on wrinkle volume at T0 before product application, 30, 120 and 240 minutes (T0.5, T2 and T4) after applications.

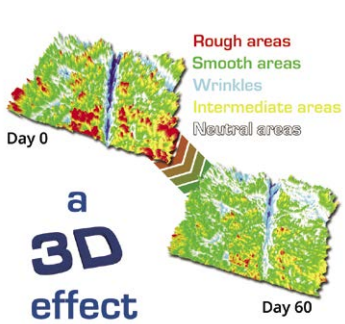

Fig. 4 3D visual representation based on integral mathematics manipulation: on 20 women aged 30 to 50. Material: Visioscan. Image analysis of parameters on wrinkle volume at D0 before product application, 15, 30 and 60 days (D15, D30 and D60) after application.

Conclusions

It becomes clearer every day that what naturally occurs in human skin is far from the outside chemical view of simply producing a bio-polymer such as HA for cosmetic use. Skin needs what skin uses, thus we need to rethink the approach to skin beauty routine.

Thanks to its know-how about bio-fermentation (HA Tech 2.0®), ROELMI HPC is able to regulate the fermentation process in order to reproduce a multiple peaks spectrum similar to the natural condition of the skin. This is a fine tuning of the standard production steps in order to modulate the efficacy as wanted. The selection of PrincipHYAL® ingredients is the result of a number of screenings on different width ranges obtained, wider or not, in order to check their activity on the skin. The four selected ingredients (Signal-10, Aurora, Cube3 and Difference) have had the best performances compared to the other ranges. Their production has been standardized and it is checked at every batch thanks to the efficacy control, an *in vitro* test showing that the selected batch works as expected.

ROELMI HPC focuses on ingredients' biological activity, the real peculiarity of an active ingredient, shown at every batch as a point of the CoA.

References

[1] *Stern R;* Glycobiology (2003); "Devising a pathway for hyaluronan catabolism: Are we there yet?"; (13): 105R

[2] *Turley EA, Noble PW, Bourguignon LYW;* J. Biol. Chem.(2002); "Signaling Properties of Hyaluronan Receptors"; (277): 4589.

[3] *Underhill CB, Toole BP;* J. Cell Biol. (1979); "Binding of hyaluronate to the surface of cultured cells"; (82): 475.

[4] *Turley EA;* Differentiation (1980); "The control of adrenocortical cytodifferentiation by extracellular matrix"; (17): 93.

[5] *Borland G, Ross JA, Guy K;* Immunology (1998); "Forms and functions of CD44"; (93): 139.

[6] *Wolny PM, Banerji S, Gounou C, Brisson AR, Day AJ, Jackson DG, Richter RP;* J. Biol. Chem. (2010); "Analysis of CD44-hyaluronan interactions in an artificial membrane system: insights into the distinct binding properties of high and low molecular weight hyaluronan"; 285 (39): 30170.

[7] *Stern R et al;* Eur. J. Cell. Biol. (2006); "Hyaluronan fragments: an information-rich system"; (85): 699.

[8] *Kothapalli D, Zhao L, Hawthorne EA, Cheng Y, Lee E, Puré E, Assoian RK;* J. Cell Biol. (2007); "Hyaluronan and CD44 antagonize mitogen-dependent cyclin D1 expression in mesenchymal cells"; (176): 535.

[9] *Kothapalli D, Flowers J, Xu T, Puré E, Assoian RK;* J. Biol. Chem. (2008); "Differential Activation of ERK and Rac Mediates the Proliferative and Anti-proliferative Effects of Hyaluronan and CD4"; 283 (46): 31823.

[10] *Pure E, Assoian RK,* Cell Signal. (2009); "Rheostatic signaling by CD44 and hyaluronan"; 21 (5): 651.

contact

Andrea Maltagliati | corresponding author
Stefania Zanzottera

ROELMI HPC srl
Via Celeste Milani, 24/26
21040 Origgio (VA) Italy

+39 0233512243
E-Mail: info@roelmihpc.com

Overall Renewal of Lipids Improves Skin Hydration and Fragrances Long Lasting

M. De Tollenaere, E. Chapuis, L. Lapierre, M. Bracq, C. Lambert, D. Auriol, A. Scandolera, R. Reynaud

abstract

Skin lipids quality and quantity are essential for an efficient barrier function and providing skin hydration. It is well described today that these parameters are affected by ageing, resulting in skin sagging, wrinkles and dryness. Due to the strong commitment of our scientists to innovate responsibly and create value, they designed a high value active ingredient named here as Vetiver extract, using a groundbreaking upcycling approach. We have verified that this unique extract was able to reactivate the skin surface lipids production, bringing skin hydration for mature skin. Among the lipids capable of modifying the skin surface, there are those from sebum and skin barrier.

We firstly demonstrated that Vetiver extract was able to increase specific lipids involved in skin hydration such as ceramides and their precursors on Reconstructed Human Epidermis and skin explants. This improvement was then evidenced at clinical level in a panel of mature volunteers with poor hydration level. The extract brought hydration through an increase of lipids content and modification of lipids conformation into the skin as measured by Raman spectroscopy.

Secondly, we demonstrated that Vetiver extract allows sebum production from human sebocytes cells lines. At the clinical level on mature volunteers, we confirmed this improvement with a significant increase of sebum production.

By influencing lipid content and structure at different levels, it was hypothesized that Vetiver extract could impact the interaction between skin lipids and volatile compounds provided by fragrance application. We demonstrated, thanks to an innovative study design, that Vetiver extract lengthens the duration of fragrance by emphasizing fragrances' heart and base notes over time.

In conclusion, we developed an upcycled active ingredient able to bring skin benefits for mature skin and boosting fragrance.

Introduction

Chrysopogon zizanioides, more commonly known as Vetiver, is a perennial grass widely cultivated in tropical regions. The plant, known to stabilize soils and limit erosion, is studied for phytoremediation and its roots contain an essential oil that is renowned in both fine fragrance and cosmetic industries.

Adding value to each part of a cultivated plant and to its by-products has become a concern for each industry with the aim of producing sustainable products. With this in mind, a cosmetic ingredient was developed from hydrodistilled roots of Vetiver that are generally considered to be a coproduct that is either burned or composted. In this study, we evaluated the biological function of this extract and especially its effect on the skin lipids including those involved in sebum production and barrier function, which can have a significant impact on the skin surface. Indeed, the skin surface lipids are recycled to ensure the integrity of the epidermal barrier and to promote skin hydration.

The lipids produced by skin cells are specific and have a major impact on the skin properties and skin surface. The keratinocytes produce the majority of lipids present into the skin. Their renewal is performed during the differentiation mechanism from the basal layer to the *stratum corneum* layer of the epidermis [1]. The *stratum corneum* contains the vast majority of skin lipids which are mainly composed of ceramides, cholesterol and long-chain free fatty acids [2,3].

The second source of lipids that can affect the skin surface are the sebum-based lipids, as a result of their hydrolipidic film forming property [4]. Indeed, sebum is released on the skin surface to promote skin protection and hydration. Sebum is produced by sebaceous glands located in the pilosebaceous unit [5]. More precisely, the sebaceous glands are composed of sebocytes that are able to deliver sebum by holocrine secretion [6].

The lipids synthetized by the keratinocytes and sebocytes are called "skin surface lipids" and together they provide a complex mixture of lipids [2,7].

Recently, it has been suggested that the skin surface lipids are able to interact with volatile organic compounds (VOCs). First, the VOCs may enhance proliferation of keratinocytes [8] and on the other hand, the composition of the skin surface lipids may play a role in body odour by being a vector for fragrances [4].

During the ageing process, an important decrease in lipid content of 30% is observed in the *stratum corneum*, which is directly correlated to a delayed barrier renewal up to one week [2]. This phenomenon can be accelerated by various factors, such as cold weather and detergent application, and all of them lead to skin dryness [9,10]. It results in a delayed renewal, inefficient corneodesmosomes degradation, a de-

crease in natural moisturizing factor (NMF) and ceramides level, and an imbalance in skin lipids composition [9,10].
Akin to the lipids from the skin barrier, the production of lipids from sebum is significantly affected by ageing. Indeed, the rate of sebum secretion decreases after 20 years of age by 23% per decade for men and 32% per decade for women [11,12].
The main objective of this study was to demonstrate that the reactivation of skin lipids synthesis and especially those involved in skin barrier and sebum accumulation using Vetiver extract could influence the skin surface. The final objective expected is to provide skin hydration. In addition, we have demonstrated that the improvement of skin hydration and lipid content can also positively influence fragrance longevity. Indeed, as volatile compounds are supposed to interact with lipids, we designed an innovative study to evaluate the long-lasting effect of the fragrance after application of Vetiver extract on the skin.

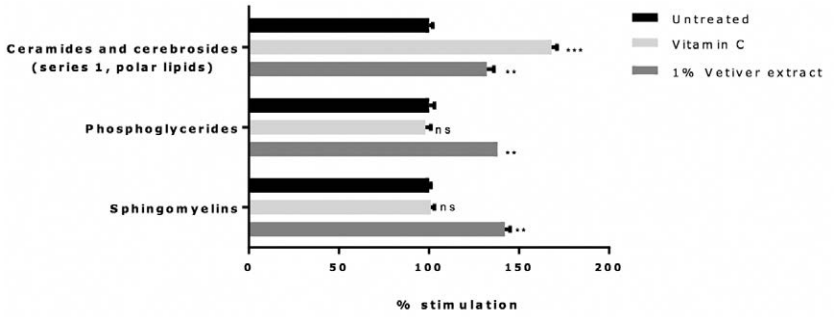

Fig. 1 Effect of Vetiver extract on [14C]-acetate incorporation in polar lipids in reconstructed human epidermis. Quantified results are expressed in % of untreated condition. Statistical analysis was performed using Student's t-test in comparison with untreated condition with *p<0.05, **p<0.01 and ***p<0.001.

Results

1. Improvement of skin lipids involved in barrier function

In vitro lipid neosynthesis in Reconstructed Human Epidermis (RHE)

In the first part of this study, we were interested in finding out whether Vetiver extract was able to reactivate the lipid synthesis in the skin barrier.
Therefore, we used an RHE model to prove the efficacy of Vetiver extract on skin lipids synthesis involved in the skin barrier. The neosynthesis of lipids was analysed by thin layer chromatography (TLC) after 7 days of treatment in presence of the extract or the positive reference, the vitamin C at 200µg mL-1. Here, we quantified the main lipid components of the epidermal barrier including ceramides and their precursors. We observed that Vetiver extract at 1% significantly induced the synthesis of sphingomyelin, phosphoglycerides, polar ceramides and cerebrosides by +42%, 38% and 32% respectively, in comparison with untreated condition after 7 days of stimulation (**Fig. 1**). These lipids promote good integrity of the skin barrier [10,13,14].
However, the treatment of the RHE with vitamin C at 200µg. mL-1, used here as positive control, resulted in a significant stimulation of polar ceramides and cerebrosides with +68% relative to the untreated condition.

Ex vivo stimulation of lipid synthesis

In order to confirm the stimulation of lipid synthesis using Vetiver extract, we performed a LipidTox™ staining on human skin explants from 3 donors after 8 days of topical application. We demonstrated that Vetiver extract at 1% significantly increased the neutral lipid content from skin explants by +29.6% (**Fig. 2**). When taken together, these data demonstrated that Vetiver extract was able to stimulate the skin barrier lipids at *in vitro* and *ex vivo* levels.

Clinical investigation of skin hydration and lipid conformation

Finally, we conducted a clinical study in order to confirm that Vetiver extract was able to improve skin barrier and related lipid content and thereby to provide skin hydration. The double blind and placebo-controlled clinical evaluation was carried out on 20 women volunteers aged 50 to 70 years old with an average age of 63 years old (±2). The volunteers were pre-selected according to their leg skin hydration level, which

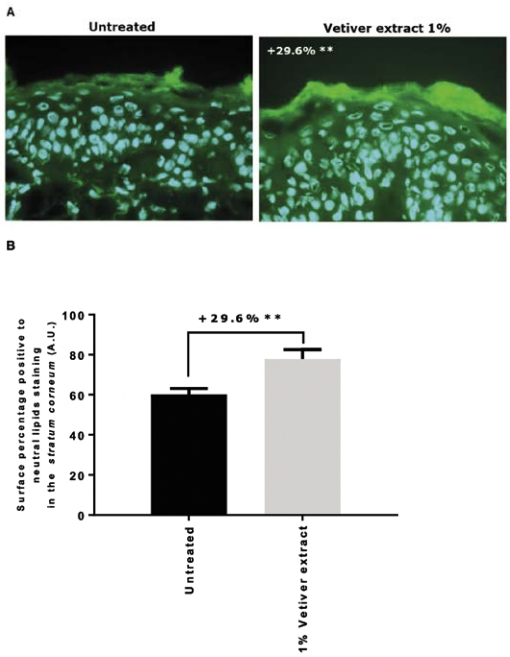

Fig. 2 Panel A: Representative pictures of 1% Vetiver extract's effect on neutral lipids synthesis (LipidTox™ staining).
Panel B: Immunofluorescence quantification of the surface percentage positive for neutral lipids staining in the *stratum corneum*.
Statistical analysis was performed using Student's t-test in comparison with untreated condition with **p<0.01.

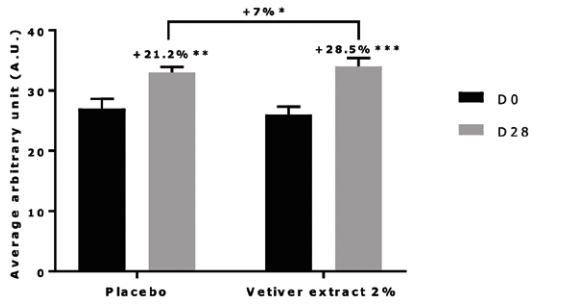

Fig. 3 Impact of products containing or not containing Vetiver extract at 2% on skin hydration after 28 days of application analysed by corneometry. Statistical analysis was performed using Student's t-test in comparison with *p<0.05, **p<0.01 and ***p<0.001.

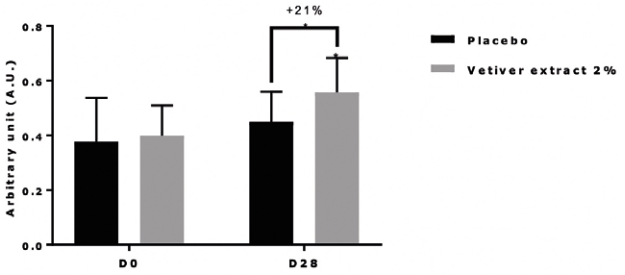

Fig. 4 Impact of products containing or not containing Vetiver extract at 2% on lipid conformation after 28 days of application analysed by Raman spectroscopy. Statistical analysis was performed using Student's t-test with *p<0.05.

had to be less than 35 (A.U.) by corneometry measurement (Corneometer® CM 825 TM, Courage & Khazaka electronics). The selected volunteers applied the products containing Vetiver extract at 2% or the placebo on their leg twice daily for 28 days. On D0 and D28, skin hydration was analysed by corneometry. Lipid conformation was evaluated by Raman spectroscopy by measuring the ratio between C-C trans and C-C gauche molecular bond. This ratio is associated with a compact state in the lipid packing [15], while a decrease is indicative of a loosening.

After 28 days, we observed a significant improvement of skin hydration with Vetiver at 2% by +28.5% compared with D0 as shown in **Fig. 3**. This effect was significantly higher than the placebo with +7% (**Fig. 3**).

This increased hydration might be explained by the improvement of lipid conformation triggered by Vetiver extract. Indeed, thanks to the Raman spectroscopy analysis we showed a significant improvement of lipid conformation by +39.6% relative to D0 with the cream at 2% Vetiver extract while the placebo didn't have any relevant impact. Again, the product at 2% Vetiver extract demonstrated a significant and more effective impact than the placebo after 28 days of application (**Fig. 4**).

The results proved that the product containing Vetiver extract stimulates moisturizing of the skin and improves the lipid conformation of the skin at the clinical level after 28 days of application.

2. Improvement of quantity of sebum lipids

In vitro lipid accumulation on sebocytes

Skin surface is also composed of lipids from sebum. Indeed, these lipids are specific and contribute to the hydration of the skin [16]. Therefore, we demonstrated that Vetiver extract was not only able to stimulate the synthesis of lipids from keratinocytes but also from sebocytes.

Human SEBO662AR sebocyte cell lines were seeded and cultured for 24 hours. Then, the sebocytes were pre-incubated for 4 days in presence or absence of the extract. After the incubation, a lipogenic mix (containing vitamin C, vitamin D3, insulin and calcium, and no androgens) was added to the medium in order to induce the lipogenesis. After 4 days of the incubation, the cells were rinsed, fixed and permeabilised. The lipid droplets contained in the cells were then labelled using a specific Bodipy® fluorescent lipid probe, labelling mainly neutral lipids. The labelling was quantified by the measurement of the fluorescence intensity normalized to the total number of cells.

The treatment of sebocytes cell line with Vetiver extract at 1% showed a significant induction of sebum production by +31% as observed by the lipid droplet formation from sebocytes after 7 days of treatment compared with the control (**Fig. 5**).

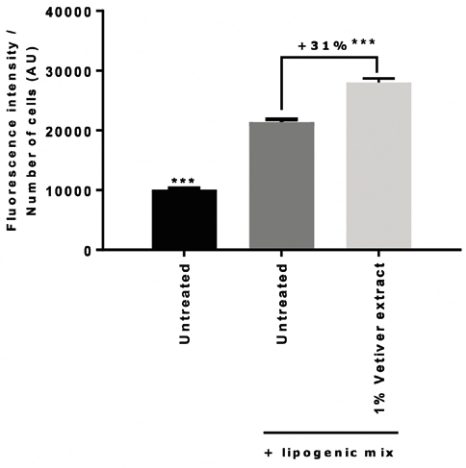

Fig. 5 Lipid accumulation analysis in sebocytes cell line after 7 days of treatment with Vetiver extract at 1%. The results were compared with a stimulated control (untreated condition with lipogenic mix) with ***p<0.001.

Clinical investigation on sebum production in elderly volunteers

To go further and confirm the previous results, we performed a clinical study focusing on sebum production on mature panel members. More precisely, the study was carried out on 30 women aged 67 +/- 2 years old who had a low facial sebum level. Indeed, a low level of sebum is known to be correlated to skin dryness [11]. Twice daily, volunteers applied the cream at 2% Vetiver extract or placebo cream in hemi face for 28 days. The sebum production was measured using Sebumeter® (SM810, Courage & Khazaka electronics) on two different areas including forehead and cheeks.

We demonstrated that Vetiver extract at 2% induced a significant increase of sebum production on

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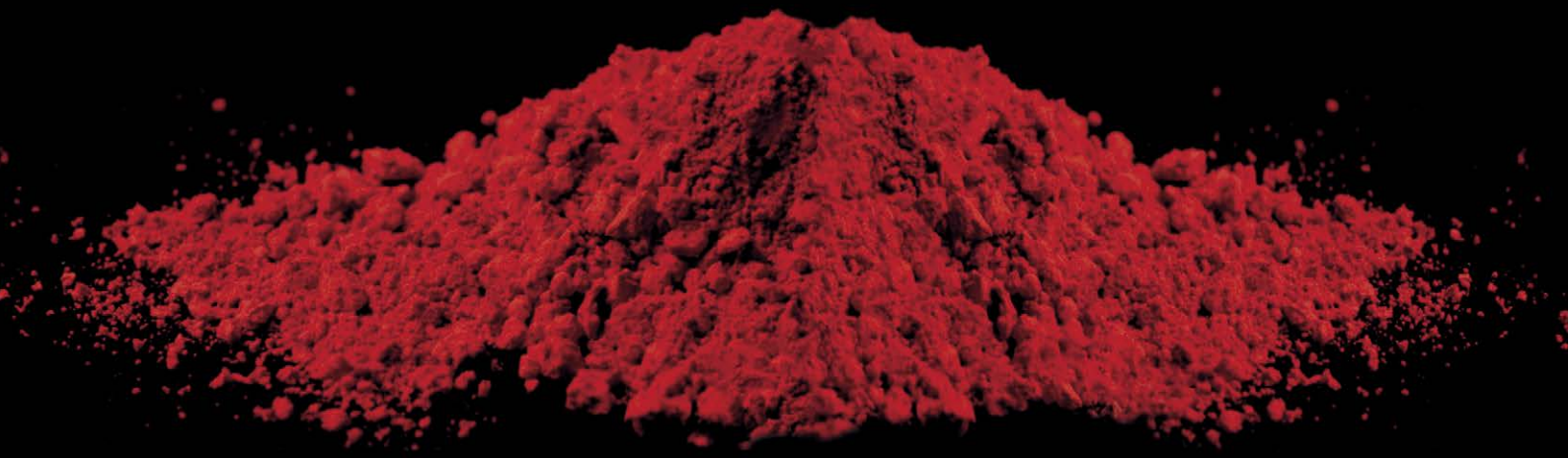

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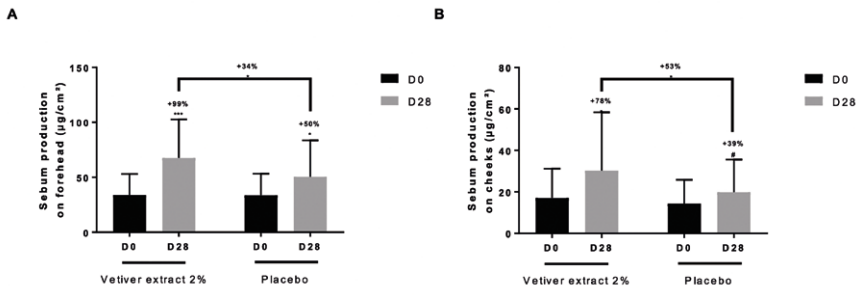

Fig. 6 Panel A: Impact of products with or without Vetiver extract at 2% on forehead sebum production after 28 days of application. Panel B: Impact of products with or without Vetiver extract at 2% on cheek sebum production after 28 days of application. Statistical analysis was performed using Student's t-test in comparison with untreated condition with *p<0.05, ***p<0.001.

forehead and cheeks in comparison with D0 by +99% and +78% (**Fig. 6**) respectively. Moreover, these inductions are significantly higher than the placebo with +34% on forehead and +53% on cheeks.

Therefore, we demonstrated that Vetiver extract at 2% is able to reactivate the sebum production that is drastically reduced on mature people.

3. Improvement of fragrance long lasting: self-assessment of olfactory intensity

By influencing lipid content and structure at different levels, it was hypothesized that Vetiver extract could interfere with fragrance. Indeed, enfleurage, an old extraction technique used by perfumers in the South of France in Grasse was based on the interaction of volatile compounds from botanical matter and animal fats [17]. On this principle, it was hypothesized that volatile compounds could be intercepted by the lipids of our skin [4, 8].

We designed an innovative study to evaluate the impact of this restructuring of skin lipids on the long duration of the fragrance. A double blind clinical study with a panel of 20 women volunteers was carried out. The volunteers applied the products containing Vetiver extract at 2% or placebo on their forearm twice daily for 28 days before the olfactory evaluation. The long-lasting effect of fragrance was measured after 1 month of product application on the forearm and the olfactory intensity was evaluated at T0, T2h and T4h after fragrance application by self-assessment.

At T0, the results showed that the intensity of fragrance seemed to be more intense on the forearm treated for 1 month with the cream containing Vetiver extract at 2% in comparison with placebo (5.58 A.U. vs 4.71 A.U.).

We observed a significant decrease of fragrance intensity on the forearm treated with placebo while those treated with 2% Vetiver extract cream maintained the fragrance intensity after 2 hours and 4 hours of fragrance application. The difference of intensity was significant compared with the placebo, by +25% and +21% at T2h and T4h respectively.

We proved that the application of the cream containing Vetiver extract at 2% for 1 month led to a significant prolongation of the fragrance's duration (**Fig. 7**).

Conclusion

Skin integrity is a delicate balance, and its maintenance requires many factors in order to play a role. It is well described today that skin lipids have a major role in maintaining skin integrity [1,2]. As previously described, different lipids are produced by the skin cells, mainly by keratinocytes during the differentiation process and by sebocytes through sebum synthesis [18].

Intrinsic ageing disrupts this integrity by slowing down the differentiation mechanism [14] and by reducing lipogenesis in sebocytes [11]. The renewal of skin lipids is disturbed and this results in dehydration of the skin and acceleration of intrinsic ageing [10,19].

Nowadays, Vetiver roots are mainly used in the perfume industry as an iconic plant and become waste once they are distilled. We started with the idea that the upcycling of exhausted Vetiver roots could bring with it a new trend of consumption in the cosmetic industry. This upcycling reminded us of the recycling loop of the skin lipids and inspired an evaluation of the Vetiver extract on the skin lipids. We demonstrated both *in vitro* and *ex vivo* that it was able to increase the quantity and the quality of lipids produced by keratinocytes and sebocytes leading to a general improvement of skin surface lipids. Then, via various clinical studies, we confirmed the efficacy of the extract on increased skin hydration and sebum content in mature women.

We thus conclude that Vetiver extract has an anti-ageing effect by promoting skin hydration, and that this improvement leads to a longer duration of fragrances on the skin.

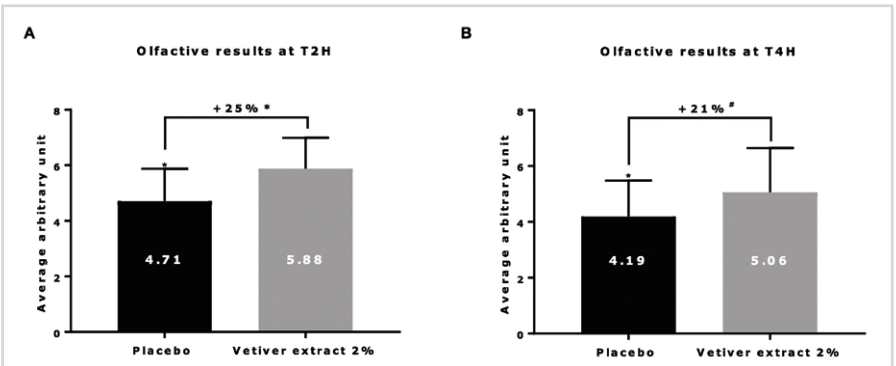

Fig. 7 Self-assessment of olfactory intensity after application of product containing or not containing Vetiver extract at 2% for 1 month with regard to Fragrance duration.
Panel A: After 2 hours. Panel B: After 4 hours. Statistical analysis was performed using Student's t-test with #p<0.1 and *p<0.05.

Bibliography

[1] *Feingold, K. R. & Elias, P. M.* Role of lipids in the formation and maintenance of the cutaneous permeability barrier. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids 1841, 280–294 (2014).

[2] *Elias, P. M. Stratum Corneum* Defensive Functions: An Integrated View. Journal of Investigative Dermatology 125, 183–200 (2005).

[3] Nishifuji, K. & Yoon, J. S. The *stratum corneum*: the rampart of the mammalian body: *Stratum corneum* in mammals. Veterinary Dermatology 24, 60-e16 (2013).

[4] *Zouboulis, C. C. et al.* Frontiers in sebaceous gland biology and pathology. Experimental Dermatology 17, 542–551 (2008).

[5] *Picardo, M., Ottaviani, M., Camera, E. & Mastrofrancesco, A.* Sebaceous gland lipids. Dermato-Endocrinology 1, 68–71 (2009).

[6] *Lovászi, M., Szegedi, A., Zouboulis, C. C. & Törőcsik, D.* Sebaceous-immunobiology is orchestrated by sebum lipids. Dermato-Endocrinology 9, e1375636 (2017).

[7] *De Luca, C. & Valacchi, G.* Surface Lipids as Multifunctional Mediators of Skin Responses to Environmental Stimuli. Mediators of Inflammation 2010, 1–11 (2010).

[8] *Groen, D. et al. In vitro* model systems for studying the impact of organic chemicals on the skin barrier lipids. Biochimica et Biophysica Acta (BBA) - Biomembranes 1838, 310–318 (2014).

[9] *Ishikawa, J. et al.* Dry skin in the winter is related to the ceramide profile in the *stratum corneum* and can be improved by treatment with a Eucalyptus extract. Journal of Cosmetic Dermatology 12, 3–11 (2013).

[10] *Rawlings, A. V. & Matts, P. J. Stratum Corneum* Moisturization at the Molecular Level: An Update in Relation to the Dry Skin Cycle. Journal of Investigative Dermatology 124, 1099–1110 (2005).

[11] *Downing, D. T., Wertz, P. W. & Stewart, M. E.* The role of sebum and epidermal lipids in the cosmetic properties of skin. International Journal of Cosmetic Science 8, 115–123 (1986).

[12] *Pochi, P. E., Strauss, J. S. & Downing, D. T.* Age-related Changes in Sebaceous Gland Activity. Journal of Investigative Dermatology 73, 108–111 (1979).

[13] *Hamanaka, S. et al.* Human Epidermal Glucosylceramides are Major Precursors of *Stratum Corneum* Ceramides. Journal of Investigative Dermatology 119, 416–423 (2002).

[14] *van Smeden, J., Janssens, M., Gooris, G. S. & Bouwstra, J. A.* The important role of *stratum corneum* lipids for the cutaneous barrier function. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids 1841, 295–313 (2014).

[15] *Choe, C., Lademann, J. & Darvin, M. E.* A depth-dependent profile of the lipid conformation and lateral packing order of the *stratum corneum in vivo* measured using Raman microscopy. The Analyst 141, 1981–1987 (2016).

[16] *Verdier-Sévrain, S. & Bonté, F.* Skin hydration: a review on its molecular mechanisms. Journal of Cosmetic Dermatology 6, 75–82 (2007).

[17] *Lawrence, B.* Essentiels oils. (1980).

[18] *Pappas, A.* Epidermal surface lipids. Dermato-Endocrinology 1, 72–76 (2009).

[19] *Krutmann, J., Bouloc, A., Sore, G., Bernard, B. A. & Passeron, T.* The skin aging exposome. Journal of Dermatological Science 85, 152–161 (2017).

contact

Morgane De Tollenaere | Skin Biology Scientist
Emilie Chapuis | Clinical Trial Manager
Laura Lapierre | Clinical Trial Technician
Marine Bracq | Laboratory Technician
Carole Lambert | Enzymatic Chemistry Project Manager
Daniel Auriol | Scientific Director Biotechnology & Fractionment
Amandine Scandolera Ph.D | Head of biological evaluation
Romain Reynaud | R&D Director

Givaudan Active Beauty
E-mail: global.cosmetic@givaudan.com

Oily Skin under Control for All

J. Comas, O. Laporta, E. Cañadas, A. Soley, R. Delgado

abstract

When dealing with oily skin, finding the suitable cosmetic product and routine can become a hard task. Besides seeking for a mattifying effect able to reduce shininess throughout the day, there is also a general concern to maintain the basic needs of hydration, which is impaired when reducing sebum levels. Therefore, a decrease in sebum content and a maintenance in skin hydration become the perfect combo for such skin types, and this is now possible thanks to Matmarine™ biotech ingredient, a marine ingredient able to control oily skin in multiethnic volunteers and in both women and men.

Benefits and negative outcomes of sebum

The main trait of oily and combination skin is an increased presence of sebum that is secreted towards the epidermis by the sebaceous glands. Although teenagers are the main group suffering from sebum raised levels in the skin, adults can also experience it, even over the age of 25. Sebum secretion is stable during decades in adults, but several factors can influence it, including hormones, which induce sebum increase during puberty and decrease during menopause. In general, sebum secretion is also higher in men, attributed to higher testosterone levels and to more pilosebaceous units.

An excess of sebum becomes a main concern, not only due to the visual impact that it has on the skin, but also because it results in clogged pores, abnormal desquamation and follicular hyperkeratinization, which are also related to acne and inflammatory processes. Nevertheless, sebum is also necessary for the skin, since it is implied in thermoregulation, maintenance of the functional epidermal barrier, hydration, skin lubrication and protection against UV exposure among others.

The production and final release of sebum onto the epidermis needs to be adequate, avoiding unnecessary levels that alter the skin and induce a dull and greasy aspect, blackheads and enlarged pores. On the other hand, when reducing sebum levels on the skin, moisture is also often impaired, leading to a dehydrated skin, which in turn produces more sebum to protect itself. Thus, in oily skin it is important to maintain the sebum levels low, while maintaining a proper skin hydration.

Controlling sebum secretion through sebocytes

The sebaceous gland cells known as sebocytes are responsible for lipid production. Once sebum accumulates in the glands, it is released to the epidermis via the hair follicles. Nevertheless, only differentiated sebocytes are involved in sebum production and for this reason, it is important to regulate sebocyte differentiation in order to control the final lipid content [1].

Several components exist that can act on sebocyte activity and sebum production, binding to specific receptors inducing either an enhancing or inhibiting effect. It is the case of the peptide hormones melanocortins, which have an important role in sebum production [1, 2].

The pituitary gland in the brain is known to synthesize propiomelanocortin (POMC), decomposing it into α-melanocyte-stimulating hormone (α-MSH) and adrenocorticotrophic hormone, which are referred as melanocortins [2, 3]. POMC was found to stimulate both sebocyte differentiation and sebum production, melanocortins increased sebum production, and specifically α-MSH showed to promote sebum secretion [1, 2, 4]. In addition, it is common for skin cells to release melanocortins and express POMC and melanocortin receptors (MCRs) [3]. Regarding sebaceous glands, the only MCRs identified are the melanocortin-5 receptor (MC5-R) and the MC1-R, both expressed in the cell surface of sebocytes [4, 5]. MC5-R is known to be only present in differentiated cells and it is a marker of sebocyte differentiation and lipid storage, directly linked to sebum production [1, 6].

Matmarine™ biotech ingredient, targeting MC5-R to decrease sebum levels

Matmarine™ biotech ingredient is a fermentation-based extract obtained from a marine microorganism (Pseudoalteromonas strain), that decreases MC5-R levels and delays sebocytes differentiation. In different ethnicities, and in both men and women, it helps improve the appearance of combination and oily skin while keeping it hydrated.

Reduction in MC5-R protein levels

Primary human sebocytes were incubated in reduced-serum medium (differentiated cells) with and without 1 mg/mL Matmarine™ biotech ingredient concentrate. MC5-R was fluorescently labeled and measured through flow cytometry. The protein levels of MC5-R were found to be reduced by 36.6%.

Decrease in sebocyte differentiation and lipid accumulation

Human sebocytes were incubated in differentiation conditioned medium (DCM) with α-MSH as an inducer, in DCM with retinoic acid (1 nM, positive control) or in DCM with Matmarine™ biotech ingredient concentrate (10 mg/mL). Non-differentiated cells were used as a control.
Differentiation of sebocytes was evaluated by measuring cellular size and granularity (inner particles which are directly correlated with the differentiation of sebocytes) through flow cytometry. Matmarine™ biotech ingredient was found to inhibit sebocytes maturation, while resembling the non-differentiated sebocytes.
On the other hand, lipid storage was also evaluated by measuring neutral lipids fluorescence. Calculations showed that the marine ingredient decreased the accumulation of sebaceous lipids up to 35.2%.

Inhibition of lipid peroxidation

Matmarine™ biotech ingredient concentrate (62 mg/mL) was incubated with small unilamellar vesicles that contained lipids from the cellular membrane. After incubation, a free radical generator was added and the induction of lipid peroxidation was measured by fluorescence through a thiobarbituric acid reactive substances (TBARS) assay, obtaining a decrease in this kind of damage up to 16.3% after the active treatment.

Microarray analysis

Human epidermal keratinocytes and sebocytes were treated separately with Matmarine™ biotech ingredient concentrate (10 mg/mL). A microarray analysis was used to detect the genes that were regulated by the ingredient.
Results demonstrated the ability of the marine ingredient to downregulate the expression of genes involved in inflammation (TNF and IL-8) in both keratinocytes and sebocytes, and it upregulated those involved in the antioxidant response (SGLC and SOD2) and in hydration (AQP9 and HAS1) in keratinocytes.

Type I collagen induction

Human dermal fibroblasts were incubated with fresh medium containing scalar dilutions of Matmarine™ biotech ingredient concentrate, while non-treated cells were used as a control. Then, well medium was collected and analyzed by an enzyme-linked immunosorbent assay (ELISA).
Matmarine™ biotech ingredient concentrate (50 mg/mL) significantly increased type I collagen synthesis up to 128.4%, which could be linked to an improved skin firmness and pore reduction.

Minimized pores and moisturized skin in men

31 Caucasian male volunteers between 19-44 years old with combination skin applied a gel containing 3% Matmarine™ biotech ingredient G on half face and a placebo cream on the other half, twice a day for 7 days. The ability of the ingredient to reduce the number of pores on the side of the nose area was measured by image analysis of standardized photographs, resulting in a 10.5% reduction after 7 days. (**Fig. 1**)
Skin moisturization was also determined in the cheekbones area by means of corneometry. The ingredient presented an instant and long-lasting skin moisturizing effect after a single application, while it also increased skin hydration by 12.4% after 7 days of use.

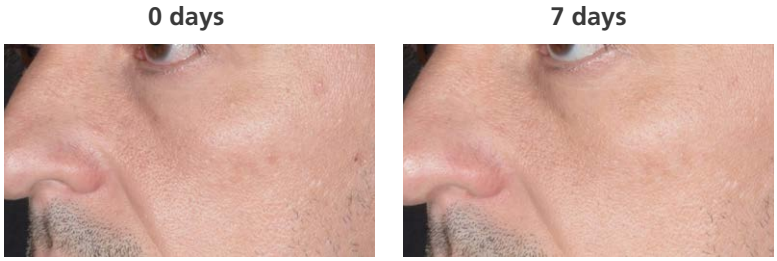

Fig. 1 Images of a volunteer before and after the active treatment.

Sebum, shininess and skin pores in Caucasian skin

20 female volunteers between 20-35 years old with oily skin applied a cream containing 5% Matmarine™ biotech ingredient on the face, twice a day for 28 days. Measurements were taken at the beginning, after 14 days and at the end of the treatment. High resolution photographs were obtained, and the number and total area of pores were calculated through a specific software.
After only 14 days of the active treatment the number and total area of skin pores decreased by 20.5% and 18.8% respectively, obtaining similar effects after 28 days. (**Fig. 2**)
Skin shininess was also evaluated in 19 subjects through digital images of the face and a reduction of 27.3% was obtained after 28 days of treatment.

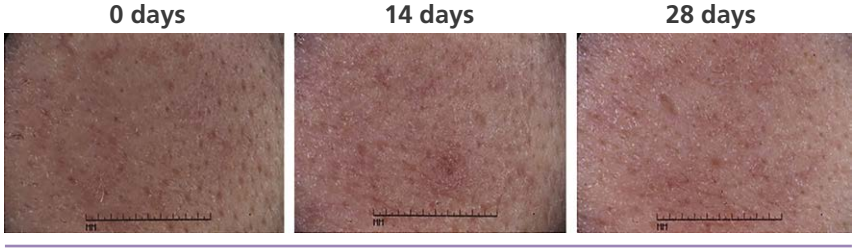

Fig. 2 Images of skin pores of a volunteer at different times.

Finally, skin sebum rate was evaluated in 20 volunteers by measuring the sebaceous secretions based on a photometric method. Results indicated a decrease in sebum rate of 9.4% after 28 days.

Sebum regulating effect on Asian skin

A panel of 20 Asian female volunteers between 20-35 years old with oily skin applied a cream with 3% Matmarine™ biotech ingredient on the face, twice a day for 28 days (**Fig. 3**).

Fig. 3 Images of an Asian volunteer before and after the treatment.

The number and total surface of follicles on the forehead were studied using a sebum-sensitive adhesive film. A decrease in these parameters means a sebum-regulating effect. Matmarine™ biotech ingredient reduced the number (9.5%) and the total area of active follicles (27.2%) after 28 days, which is related to a minor sebum production.

Mattifying efficacy in a BB cream along the day

A group of 22 Caucasian female volunteers with combination skin applied a BB cream containing 2% of the marine ingredient on a hemi-face and a placebo BB cream on the other. Through a light reflectance principle, the skin shine was evaluated immediately after the application, and 2 and 8 hours later. An immediate reduction of 28.3% in skin gloss was observed after the treatment with Matmarine™ biotech ingredient, while this mattifying effect was maintained throughout the day.

Conclusions

Matmarine™ biotech ingredient is the ideal ingredient to include in skin care formulations intended to reduce excessive sebum production and minimize the undesired features of oily and combination skin, while maintaining a proper skin hydration. It can be incorporated into products not only for teenagers but also for adult women and men to decrease skin sebum, shininess and pores, and to also minimize inflammation and improve hydration. It is also a great ingredient to incorporate into BB creams as a way to potentiate multifunctional formulations.

Bibliography

[1] *Zhang L, Li WH, Anthonavage M, et al.* Melanocortin-5-Rezeptor und Sebogenese. Eur J Pharmacol. 660: 202-206, 2011.

[2] *Smith KR, Thiboutot DM.* Talgdrüsenlipide: Freund oder Feind? J Lipid Res. 49: 271-281, 2008.

[3] *Böhm M, Luger TA, Tobin DJ.* Melanocortin-Rezeptor-Liganden: Neue Horizonte für die Hautbiologie und klinische Dermatologie. J Invest Dermatol. 126: 1966-1975, 2006.

[4] *Zouboulis CC, Xia L, Akamatsu H, et al.* Das Modell der menschlichen Sebozytenkultur liefert neue Erkenntnisse über die Entwicklung und Behandlung von Seborrhoe und Akne. Dermatologie. 196: 21-31, 1998.

[5] *Deplewski D, Qin K, Ciletti N, et al.* Einzigartiger Modus der lipogenen Aktivierung in präputialen Sebozyten der Ratte. J Nutr Metab. 2011: 163631, 2011.

[6] *Zhang L, Li WH, Anthonavage M, et al.* Melanocortin-5-Rezeptor: Ein Marker für die Differenzierung menschlicher Sebozyten. Peptide. 27: 413-420, 2006.

contact

Júlia Comas
Olga Laporta
Elena Cañadas
Albert Soley
Raquel Delgado

Lipotec S.A.U.
Gavà | Spanien

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Nature-based Rheology Modifiers and Emollients for Alcohol-based Formulations

T. Nuutinen, E.-J. de Feij, X. Qu, A. Druffner

abstract

Today, hand sanitizers formulated with >60% alcohol are more important than ever to kill germs on hands when soap and water are not available. The choice of ingredients for alcohol-based formulations is critical to deliver an efficacious, stable product with pleasing aesthetics. As consumers are increasingly choosing products with ingredients that originate in nature and are based on renewable resources, nature-based rheology modifiers and emollients are valuable for alcohol-based formulations.

In recent studies, cellulose-based thickeners and emollient esters were evaluated in prototype hand sanitizer formulations with 70% ethanol for rheological properties, clarity, electrolyte stability and sensory properties. Formulations tested containing hydroxypropyl methylcellulose (HPMC) and hydroxypropylcellulose (HPC) were compared to acrylate-based formulations. The results show key differences between performance of the formulations with the cellulose-based thickeners showing improved rheology properties in the presences of salt. In consumer tests, the differences in sensory were also noted. For example, panelists noted better moisturization and spreadability with HPC and HPMC. Thus, the choice of ingredients is an important consideration in formulating hand sanitizers, both for sanitizing performance and for positive sensorial experience.

Hand sanitizer use is on the increase. For example, almost 20% of consumers in Germany are using hand sanitizers more often and 59% of consumers in the U.K. report using hand sanitizer more often [1,2]. Typically, hand sanitizers are clear to translucent, hydroalcoholic solutions, with alcohol content of at least 60%. The formulations often contain rheology modifiers and skin conditioning agents to enhance the user experience. Various types of rheology modifiers have found use in hand sanitizer formulations. Traditionally, synthetic acrylate thickeners, such as carbomers, have been used. However, as consumers are attracted to ingredients originating in nature and give good sensory properties, there is increasing use of cellulosic thickeners, including hydroxyethylcellulose (HEC), hydroxypropycellulose (HPC) and hydroxypropyl methylcellulose (HPMC).

Cellulosic thickeners have origins in nature as they are derived from cotton fibers and/or trees [3]. The cotton- and wood-cellulose is then reacted with various functional groups to produce several variants. Chemically, cellulose ethers include carboxyalkyl, hydroxyalkyl, alkyl and alkylhydroxyalkyl groups attached to a cellulose backbone by an ether bond. Cellulosic thickeners, including HEC, HPC and HPMC, have been used very successfully as rheology modifiers, foam enhancers and film formers for various applications in personal care, including hand sanitizers.

The selection of the rheology system for a hydroalcoholic hand sanitizers, requires consideration of the alcohol compatibility; the desired viscosity of the final product; the processing and packaging requirements and limitations as well as the consumer's experience. Consumers expect a product to be easy-to-use, dry quickly, appear clear on hands, have pleasant or no odor, feel good, and leave no pilling or residue on hands. Although the primary role of the rheology modifier system is to thicken a formulation, the rheology modifier must not impact the delivery of the alcohol which is the active ingredient in a hydroalcoholic hand sanitizer. And today, formulators must also consider the sustainability and sourcing of an ingredient prior to selection. In this study, the properties of cellulosic thickeners on hydroalcoholic hand sanitizers were studied.

Materials and Methods

Nature-based rheology modifiers and emollients

A range of cellulose-based rheology modifiers and emollients derived from nature were selected for evaluation in hydroalcoholic systems (**Tab. 1** and **2**). All the cellulose rheology modifiers are nonionic.

Formulations

The formulations, with 70 wt% ethanol, are shown in **Tab. 3** and **4**. For the sensory and hydration studies, the formulations in **Tab. 3** are tested. Formulations A and B use a cellulose-derived rheology modifier while formulation C contains a traditional acrylate rheology modifier (INCI: Acrylates/C10-C30 Alkyl Acrylate Crosspolymer). The viscosity and clarity of each

formulation is noted. The formulation in **Tab. 4** is used to study the impact of various emollients on formulation viscosity and sensory attributes.
To prepare the formulations with the HPC and HPMC, always disperse polymer in alcohol. After 10 minutes, add water and mix until full viscosity develops. Water is required for full hydration. Neutralization is not required.

Antimicrobial Activity

Studies are conducted using ASTM E2315, "Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure." *Staphylococcus aureus* (ATCC 6538) is used as the test organism.

Viscosity Measurements

Viscosities of all formulations is measured using Brookfield RVT viscometer. The spindle, speed, time and temperature are noted in the results.

Skin Hydration Evaluation

The Corneometer™ CM825 skin hydration instrument is used to measure skin hydration initially and after 1, 3 and 6 hours. This widely used instrument measures the hydration level of the skin surface, mainly the stratum corneum.
Ten Shanghai residents participated in the randomized, double blind and non-treatment control test. Testing is conducted using 100 µL of hand sanitizer on the inside of each forearm with three 3 cm x 5 cm zones marked using a dermatological pen. The statistical difference of the data is evaluated using Student's T test, one-tailed, method.

Sensory Consumer Panel Test

Formulations are evaluated using a double-blind randomized contrast test with 11 sensory evaluation experts. The formulations (200 µL) are applied to both inside of forearm or on back of hand and rubbed with fingers on 5 cm diameter circles. Volunteers refer to a standard scale to grade the appearance, rub-out application and the after-feel at 0 and 5 minutes. Formulations are evaluated in matched pairs.

INCI	Source	% Natural origin*	Alcohol compatibility	Typical grades (supplier)
Hydroxyethylcellulose (HEC)	cellulose	>54%	60-65%	HR/HHR (Ashland)
Hydroxypropylcellulose (HPC)	cellulose	>39%	100%	H/M (Ashland)
Hydroxypropyl methyl cellulose (HPMC)	cellulose	>80%	70%	E10M (Ashland)

*according to ISO 16128:2:2017

Tab. 1 Cellulose-derived rheology modifiers

INCI	Source	% Natural origin*	Clarity in alcohol	Typical grades (supplier)
C12-C15 Alkyl Lactate	lactic acid	31%	clear	Ceraphyl™ 41 ester (Ashland)
Cetyl Lactate	lactic acid	24%	clear	Ceraphyl™ 28 ester (Ashland)
Lauryl Lactate [and] Myristyl Lactate [and] Cetyl Lactate	lactic acid	29%	clear	Ceraphyl™ 31 ester (Ashland)
Myristyl Lactate	lactic acid	23%	clear	Ceraphyl™ 50 ester (Ashland)

*according to ISO 16128:2:2017

Tab. 2 Alcohol-soluble emollients

Ingredient INCI	Formulation (w/w %)		
	A	B	C
Aqua/Water	27.50	27.20	28.13
Acrylates/C10-C30 Alkyl Acrylate Crosspolymer	–	–	0.30
Hydroxypropylcellulose (H grade)	1.00	–	–
Hydroxypropyl methylcellulose (E10M grade)	–	1.30	
Alcohol (ethanol)	70.00	70.00	70.00
Glycerin	1.50	1.50	1.50
Aminomethyl Propanol	–	–	0.07
Total	**100.00**	**100.00**	**100.00**
Viscosity, Brookfield RVT, spindle 6, 10 rpm for 1 minute @ 25°C (cP)	3,500	1,300	12,000
Clarity (% transmission @ 600 nm)	>90%	>90%	>90%

Tab. 3 Model hand sanitizer formulations for hydration and sensory studies

Ingredient INCI	Formulation (w/w %)
	D
Alcohol (ethanol)	72.90
Hydroxypropyl methylcellulose (E10M grade)	1.50
Aqua (Water)	23.10
Emollient	1.00
Glycerin	1.50
Total	**100.00**

Tab. 4 Prototype hand sanitizer formulation for emollient study.

Results and Discussion

Effect of rheology modifier on antimicrobial efficacy

The most important performance attribute of a hydroalcoholic hand sanitizer is to kill germs on hands. The rheology modifier should not impact the efficacy of the active ingredient. The antimicrobial activity of the formulations in **Tab. 3** with 60% ethanol (minimal requirement) were tested by liquid suspension time-kill method. The results show the concentration of bacteria decreased from 2.40E+07 CFU/ml to less than 10 CFU/ml after 30 seconds contact time for all samples, including the control without a rheology modifier. Based on these results, the antimicrobial efficacy of hand sanitizer is not affected by the cellulose rheology modifiers.

Impact of electrolytes on rheology

As we use our hands during the day, it is not uncommon for palms to sweat especially when excited or nervous. And sweating while wearing single-use gloves is unavoidable. Although sweat is primarily water, it also contains electrolytes. Traditionally, hydroalcoholic hand sanitizers have been thickened with acrylate polymers. However, a drawback of acrylate- based formulations, in addition to the need for neutralization, is poor electrolyte tolerance. These formulations can lose structure, melt or break rapidly when applied to sweaty palms and hands. In one study [4], the viscosity profiles of hand sanitizers formulations containing 65% ethyl alcohol and 0.25% carbomer with 0.1% aminomethyl propanol (AMP) for neutralization; 1.5% HPMC or 1.0% HPC were evaluated using texture analyzer with 60 mm cone and plate at 25°C. The formulations were evaluated as is and dosed with 50 ppm sodium chloride solution to mimic the sweat on the skin. The results (**Fig. 1**) show the viscosity decrease of the carbomer/AMP formulation. This illustrates the effect that a small amount of salt has on formulation viscosity. The formulation containing HPMC and HPC demonstrate excellent electrolyte tolerance and the formulation with HPMC shows the least response to shear rate changes of all three formulations.

Influence emollients on formulation viscosity

Emollients are used to soften and smooth the skin. Thus, alcohol-soluble emollients are added to hydroalcoholic formulations in order to enhance the sensory appeal. However, emollients can also work synergistically with cellulose ether rheology modifiers to build viscosity. To evaluate the impact of emollients on formulation viscosity, the formulation in **Tab. 4** was prepared using 3 different emollients. The emollient studied were cyclomethicone, lauryl/myristyl/cetyl lactate blend and C12-C15 alkyl lactate. Formulation viscosity was measured after 24 hours using a Brookfield LVT viscometer with spindle 64 at 30 rpm at 25°C. The results (**Fig. 2**) show a higher viscosity is achieved using the lauryl/myristyl/cetyl lactate blend compared to the C12-C15 alkyl lactate or cyclomethicone. Hence, formulators can consider the use of emollients to enhance formulation viscosity as well as sensorial properties.

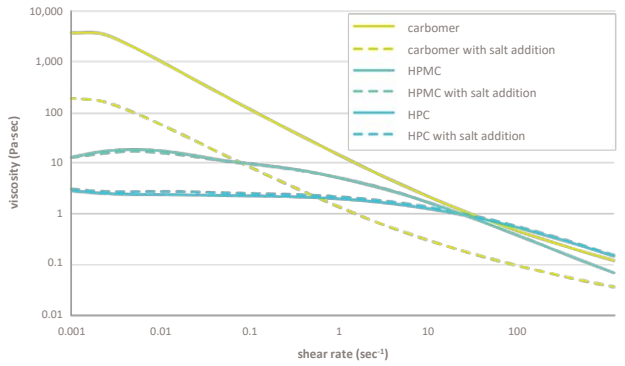


Fig. 1 Viscosity profiles for three formulations with (open) and without sodium chloride salt (closed).

Effect of rheology modifiers on skin hydration

Consumers understand that the continued use of alcohol-based hand sanitizers dries out skin. Alcohol causes denaturation of stratum corneum proteins and depletes the lipid barrier of skin [5]. As a result, many hydroalcoholic hand sanitizer formulations will contain glycerin or a humectant or other ingredients to condition and soothe skin. However, the choice of rheology modifier may also influence skin hydration.

Hand sanitizer prototypes (formulation A and C) were evaluated for skin hydration. The formulations differed by the type of rheology modifier used to thicken the 70 wt% alcohol. The results (**Fig. 3**) indicate a statistical difference in hydration after 1 and 3 hours compared to baseline with formulation A over formulation C and a directional improvement after 6 hours. Formulation A contains HPC which acts as a film former.

In addition, the contact angle of water on the dried films was measured. The contact angle for the HPC film is 57 degrees while the contact angle for the acrylate polymer film is 104 degrees. These results indicate the HPC film is also more hydrophilic than the acrylate polymer film. Thus, the choice of rheology modifier can impact skin hydration and HPC should be considered to enhance the condition of skin.

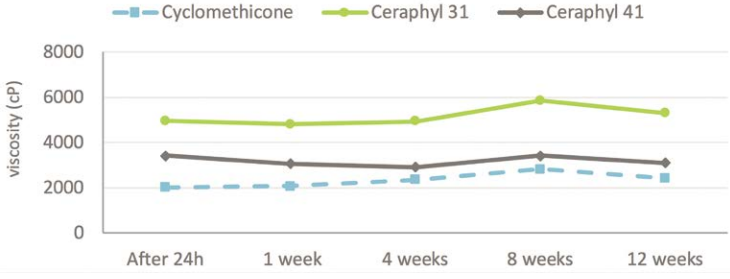
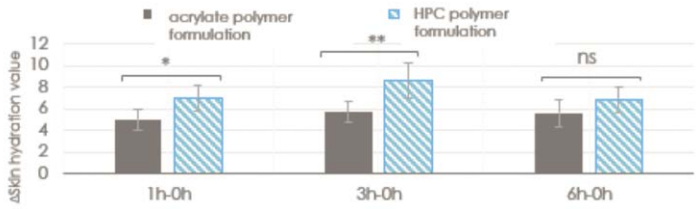
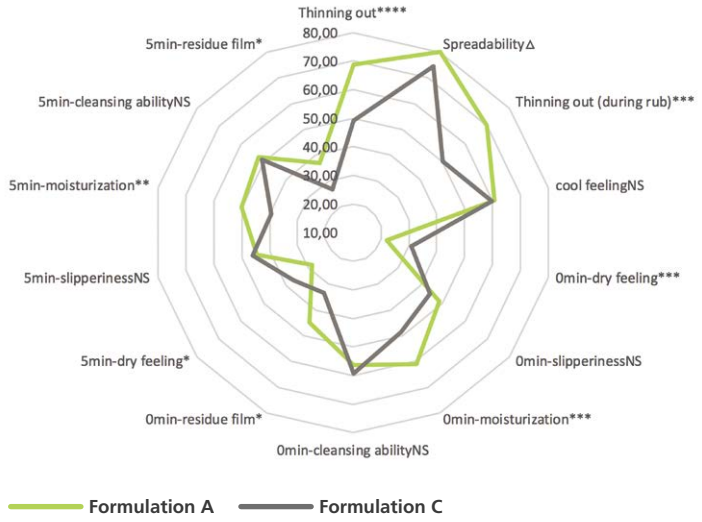


Fig. 2 Viscosity profiles of hand sanitizer prototypes with various emollients.



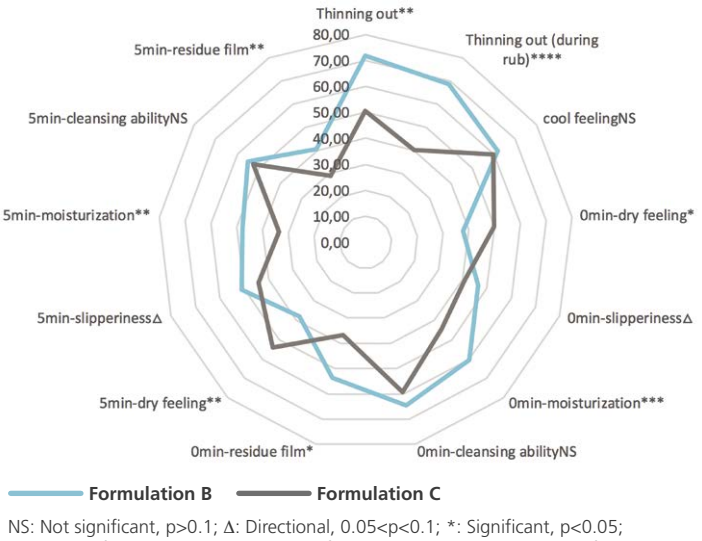
NS: Not significant, p>0.1, *: Significant, p<0.05, **: Very significant, p<0.01

Fig. 3 Skin hydration results of hydroalcoholic formulations with different rheology modifiers using Corneometer instrument.



NS: Not significant, p>0.1; Δ: Directional, 0.05 <p<0.1; *: Significant, p<0.05; **: Very significant, p<0.01; ***: Highly significant, p<0.005; ****: Extremely significant, p<0.001

Fig. 4 Sensory evaluation results of model hand sanitizer formulations: HPC vs acrylate thickeners.



NS: Not significant, p>0.1; Δ: Directional, 0.05<p<0.1; *: Significant, p<0.05; **: Very significant, p<0.01; ***: Highly significant, p<0.005; ****: Extremely significant, p<0.001

Fig. 5 Sensory evaluation results of model hand sanitizer formulations: HPMC vs acrylate thickener.

Role of rheology modifiers on sensorial properties

Sensory appeal of a formulation is an important consideration for continued use by consumers. It is well discussed in the literature that the choice of rheology modifier can impact sensory properties of a formulation. To further understand the impact of nature-based cellulose ethers on the sensorial properties of hand sanitizers, a consumer panel study was conducted. The results (**Fig. 4** and **5**) show the HPC- and HPMC-containing formulations (formulation A and B) surpassed the traditional acrylate-based formulation (formulation C) in almost every evaluation dimension, including hydration, feel and spreadabilty. This study indicate consumer preference for the formulations with nature-based thickeners.

Conclusions

Cellulose-derived thickeners are well suited for hand sanitizer formulations. As they are compatible with alcohol and do not require neutralization, they are easily formulated. The resulting formulations have good electrolyte tolerance for stability with sweaty skin and superior sensorial properties.

Acknowledgements

The authors would like to acknowledge *Van Nguyen* and *Cassie Guo* for their contributions. Additionally, the authors would like to thank *Coralie Alonso* and *Surya Kamin* for review of this article.

References

[1] Mintel, "Covid-19 impact-on-German-consumers," 2020.

[2] Mintel, "Patent Insights: Hand Sanitizers," July 2020.

[3] *R.C. Pettersen*, "The chemical composition of wood" in The Chemistry of Solid Wood, Ed. R.M. Rowell, Advances in Chemistry Series 207, Washington, D.C.: American Chemical Society; 1984: Chapter 2.

[4] *S. Thiewes, J. Modi, J. Williamson, K. Gaughan, J. Sosnowik,* "Effect of Rheology Modifiers on the Rheological and Sensory Behavior of a Series of Hand Sanitizers," presented at Society of Cosmetic Chemists Technology Showcase, 2011.

[5] WHO Guidelines on Hand Hygiene in Health Care First Global Patient Safety Challenge Clean Care Is Safer Care Geneva: World Health Organization; 2009.ISBN-13: 978-92-4-159790-6

contact

Tuttu Nuutinen | EMEA technical services manager, personal care
Eric-Jan de Feij | senior staff scientist, personal care
Ashland Industries Netherlands, BV. | Zwijndrecht | Netherlands

Xin Qu | global R&D lab manager, personal care
Ashland (China) Holdings Co., Ltd. | Shanghai | P.R. China

Ann Druffner | marketing director, home care
Ashland, Inc. | Bridgewater | NJ USA

E-Mail: tnuutinen@ashland.com

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Formulating W/O Emulsions: Features and Solutions

W/O Emulsions Meet Market Trends

W/O (water-in-oil) emulsions are becoming more and more preferred in the personal care space, particularly regarding color cosmetics and sun care.

In color cosmetics, W/O foundations offer better water resistance, long-wear, and high coverage – all the performance qualities a consumer desires – compared to O/W (oil-in-water) formulations. And though W/Si (water-in-silicone) formulations may offer great performance and texture, they do not qualify as natural makeup, making them a less desirable option among younger shoppers.

When it comes to sun care, consumers are moving towards mineral options. For example, sunscreen launches containing zinc oxide increased by 45% from 2014-15 to 2018-19 in North America [1]. Zinc oxide is easier to formulate in a W/O than an O/W system. In fact, most inorganic sunscreens are either anhydrous or W/O formulations.

However, formulating can be easier said than done, as there are many challenges that come with making a stable W/O product. W/O systems are more sensitive to other ingredients and require more energy in their processing to ensure stability. There are two key factors to consider when it comes to W/O formulation: oil polarity and electrolyte addition, which may not have any influence or not even be necessary for O/W formulations.

Oil Polarity

The selected emulsifier has distinctive characteristics that will help determine the profile of the other ingredients. One trait to consider, that is often overlooked, is polarity.

If a more polar emulsifying agent is used, then the oil phase should consist of more polar oils. Some medium to high polarity oils include C12-15 alkyl benzoate, caprylic/capric triglycerides, octyldodecyl myristate, castor oil, and coco-glycerides, among countless others.

On the other hand, if the emulsifier is not very polar, then the oil phase should be more comprised of apolar oils, such as alkanes like squalane, or undecane, tridecane, or silicones if preferred.

Electrolytes Addition

The stability of W/O emulsions can be improved by adding a proper concentration of inorganic salts, such as $CaCl_2$, NaCl, $MgCl_2$, and $MgSO_4$, in the aqueous phase [2]. Adding salts to a W/O emulsion inhibits Ostwald ripening (**Fig. 1**), which is where smaller droplets diffuse and deposit themselves onto larger droplets.

Inorganic salts can also lower the interfacial tension at an oil-water interface, thereby improving stability against coalescence and sedimentation [2] (**Fig. 2**).

Attractive forces exist within the dispersed phase and can increase their collision frequency [2]. This can lead to either coalescence or sedimentation. However, by adding electrolytes to the system, it is possible to lower the attractive forces between water droplets to ensure stability.

Needless to say, electrolytes are absolutely necessary to ensure a stable formula, maintaining the smaller droplet sizes in a consistent, even dispersion.

Emulium® Illustro, the Solution for Natural and Stable W/O Emulsions

Emulium® Illustro is Gattefossé's gold-winning, naturally derived, high performing, W/O emulsifier that makes it possible to create a natural, stable, and sensorial make-up, sun care, or skincare product.

This ingredient demonstrates superior performance and stability compared to other emulsifiers on the market. Compatibility with cosmetic ingredients – emollients, film formers, polymers, pearlescent agents, powders, stabilizers – is excellent. Flexible, easy to use, cold processable, it enables

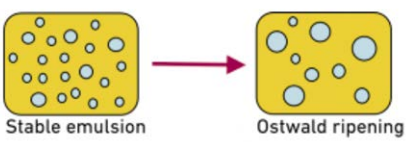

Fig. 1 Representation of the Ostwald ripening.

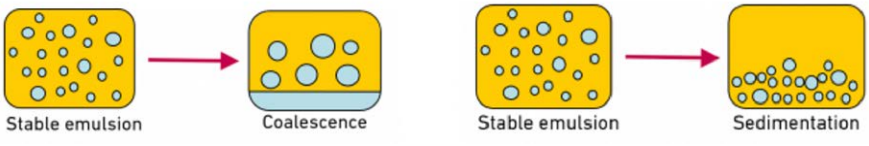

Fig. 2 Representation of coalescence and sedimentation.

the formulation of ultra-fluid to thick textures without any co-emulsifier.

Emulium® Illustro (**Fig. 3**) has a very large polar head group and long lipophilic chains that also have polar bonds, as seen by the hydroxide and ester groups.

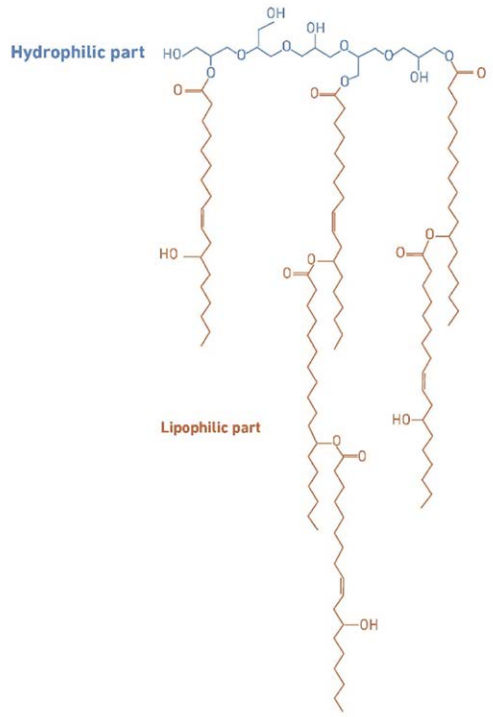

Fig. 3 Main chemical structure of Emulium® Illustro.

When formulated, the emulsifier forms a W/O emulsion. The polyglycerol-6 polar head can be found in the water phase, and the 12-hydroxystearic acid and ricinoleic acid long carbon chains extend into the oil phase, bringing stability to the emulsion by steric repulsion. In a formulation containing pigments, the long carbon chains entrap the pigments, holding them in a homogeneous stable but non-rigid dispersion.

Because of its polar properties, Emulium® Illustro is more compatible with medium to high polarity oils. But since this ingredient is highly compatible and flexible, it is possible to incorporate apolar oils at certain percentage levels. It is also compatible with salts, and more specially with 2% $MgSO_4$ or a combination of 1% of $MgSO_4$ and 1% of NaCl, making stable formulas over time.

Using a patented technology based upon polyglycerol esters, Emulium® Illustro perfectly meets consumer expectations in terms of naturality and formulators' requirements for performance and flexibility.

References

[1] Mintel GNPD

[2] *Qiaomei Zhu, Yijun Pan, Xin Jia, Jinlong Li, Min Zhang, Lijun Yin.* Review on the Stability Mechanism and Application of Water-in-Oil Emulsions Encapsulating Various Additives. (2019)

contact

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Consortium ALT-SPF

Interview with Jürgen Vollhardt, Global Head of Science & Promotion Sun Care, DSM, Member of ISO TC217/W7 for SNV and Uli Osterwalder, Principal and Owner, Sun Protection Facilitator GmbH, Chair of ISO TC217

Recently, a call for expression of interest to join the ALT-SPF consortium was launched. Can you explain in short words what this is all about?

J. Vollhardt: The ALT-SPF consortium aims to gather a critical mass of suncare industry companies and alternative test developers around the world. With this initiative, we want to combine the resources of our industry to realize a long-cherished wish to characterize new alternative SPF methods sufficiently in terms of accuracy (trueness and precision) in comparison to the *in vivo* SPF as defined by the ISO 24444 gold standard. Alternative measurements should neither over- nor underestimate the SPF values. This will allow bodies such as ISO to validate such methods and recommend them for general or specific use. We also want to give all relevant alternative methods a chance to compare themselves with the Gold Standard. The call of expression of interest is the first step in bringing all important actors together. Other important actors, such as *in vivo* test institutes, will be involved in a second step.

In your opinion, what are the weaknesses of ISO standard 24444:2019?

U. Osterwalder: First of all, the SPF *in vivo* test measures a very important number that characterizes sunscreen performance on human skin. The SPF test is close to the actual use of the products. The measured protection against sunburn (erythema) is also meaningful in terms of protection against skin cancer. Therefore, new methods should be as close as possible to the values of this gold standard. However, today's test is lengthy and it can take up to several weeks to get the result, depending on the amount of test results at the testing institute. This hardly helps a sunscreen manufacturer during development. So, the goal of the ALT-SPF consortium is also to have something faster. In addition, the radiation dose delivered by an alternative method should be negligible.

Who would you like to reach with the ALT-SPF project?

J. Vollhardt: The ALT-SPF consortium is aimed at the entire sunscreen industry and those who are involved in it. First and foremost, the users of the SPF claim, i.e. the sunscreen manufacturers who have an interest in how the SPF will be measured in the future. But it is also an interesting topic for suppliers of UV filters and ingredients in sun protection, such as DSM. We will of course also involve test institutes with conventional or alternative methods, as well as Sun Care experts who can and want to contribute. We do not want to miss an interesting method with potential. And for the alternative methods it is a unique chance to demonstrate their performance and find global recognition.

Jürgen Vollhardt

Isn't there already data showing a match?

U. Osterwalder: Of course, we assume that there is and hope that the method developers will be able to show such data. A lot has been published about the different methods, for example about *in vitro* transmission, *in vivo*/*in vitro* Hybrid Diffuse Reflectance (HDRS) or *in silico* simulation. But it turns out that the devil is always in the details. There are various sources of error, which sometimes cancel each other out randomly. But this coincidence can also work against a method. But consumer safety and the success of a method should not be based on chance. Therefore, a test procedure is needed that clearly checks the weaknesses of a method and does not just test degrees of error in a generalized way, as the usual correlation diagrams do. For validation purposes it needs to be known where the differences between gold standard and alternative method actually originate from. There are nowadays statistical procedures available that can separate the different sources of bias from each other and quantify them.

What impact do you expect from the results of the ALT-SPF project?

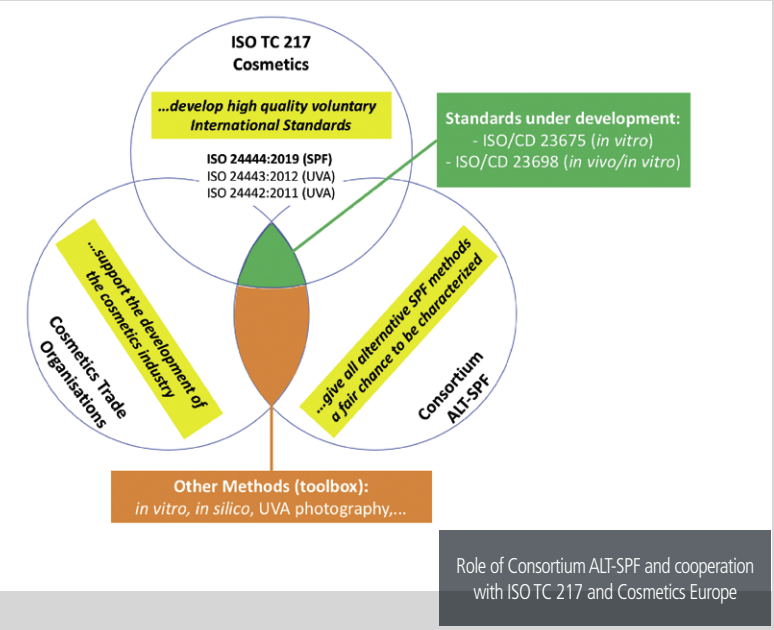
U. Osterwalder: The characterization of alternative methods relative to the gold standard will deliver data to enable a validation process defining the scope for which the alternative methods would work. For this criteria need to be agreed on. A replacement of the good old *in vivo* method could make development faster as it allows for in between checkpoints. Market surveillance by authorities could also utilize the new methods more broadly.

How can interested partners contribute to the project?

J. Vollhardt: There will be a lot of knowledge necessary, e.g. about different types of samples, specific needs of the industry and also legal aspects of the consortium that require expertise. Currently, experienced method validation statisticians are already on board and suggested an experimental design together with the analysis of the

Uli Osterwalder

data. That needs to be further worked on. The sophisticated experimental design in the end will be delivering detailed features of the alternative methods, but it still needs to be very efficient. Nevertheless, we expect significant testing efforts. To finance the whole project, we need more sponsors. We are thinking especially of sunscreen manufacturers. The test institutes will make their contribution in the form of measurements. Of course, we also welcome other important stakeholders that want bring forward this activity. Many experts are already involved in ISO TC217 WG7 (Sun Protection). The ALT-SPF consortium is planning a formal liaison with ISO in order to work even closer together, as e.g. Cosmetics Europe has been doing for a long time (**see Figure**).



Role of Consortium ALT-SPF and cooperation with ISO TC 217 and Cosmetics Europe

Where can you find more information about the project?
J. Vollhardt: The central source of information is currently our "Consortium ALT-SPF" webpage: www.alt-spf.com where the call for expression of interest to join the consortium is open until 1 September. There you will find a letter for participation and a form to fill in in which way you want to support the consortium e.g. as a sponsor and/or through in-kind contribution.

What is the role of the ALT-SPF Consortium with regards to ISO?
U. Osterwalder: ISO develops international standards on a voluntary basis. The experts come mainly from industry, but also from other stakeholders such as authorities or consumer organizations. The effort is paid for by everyone. A well-founded validation, which is in everyone's interest, means an extra effort. This is where the ALT-SPF consortium comes in. The Consortium ALT-SPF wants to ensure that the characterization is carried out according to scientific standards and that every relevant alternative method gets a chance. The resulting data will be configured to allow for validation by interested authorities or standardization bodies. The figure illustrates this role.

What is the timetable?
J. Vollhardt: The ALT-SPF consortium is now in the formal setup phase. Once the Consortium Agreement is signed, the samples will be distributed to the testing institutes and the actual testing phase can begin. This will happen in the last quarter of 2020. The tests are scheduled to last six months. This means that in the second quarter of 2021 the results can be summarized in a report. They will then be published in a scientific journal, i.e. in about a year's time, if everything goes according to plan.

Why should a personal care company become a sponsor the ALT-SPF consortium?
J. Vollhardt and *U. Osterwalder*: As a personal care company sponsoring this initiative, you:

- get access to a huge amount of data on the characterization of alternative SPF test methods
- will get to know the performance of the new methods in detail and for different product categories firsthand and can make your technology selection for your claiming needs
- can interact with ISO experts working on the alternative methods
- can spell out your expectation regarding bias and precision
- will understand why the characterization method would withstand court challenges as it will be done according to the latest technology in statistics and various norms published or in preparation.
- will help providing the industry with a fast and reliable method that will also support your development process better
- will support the in-depth investigation of the methods down to sample specific bias, their variation features relative to *in vivo* and that way defining properly the applicability and the scope of the new methods.

This interview is the personal opinion of the interviewed persons and not an official statement of the ISO committee.

www.spf-osterwalder.com www.dsm.com

CLARIANT

Illuminating Silver Hair Nectar | gCCH0153.4

Phase	Raw Material	INCI	Function	%
A	Water	Aqua	Diluent	Ad 100
	Disodium EDTA	Disodium EDTA	Chelating agent	0.10
	Polyglykol 200	PEG-4	Humectant	1.00
B	**Hydroxyethylcellulose**	Hydroxyethylcellulose	Thickening agent	0.50
C	**Plantasens® Emulsifier HE20**	Cetearyl Glucoside (and) Sorbitan Olivate	Emulsifier	3.00
	Plantasens® Flash 100	Tridecane (and) Pentadecane	Emollient	4.00
	Plantasens® Abyssinian Oil	Crambe Abyssinica Seed Oil	Emollient	3.00
	Genadvance® Life	Polyquaternium-116	Hair conditioning agent	2.00
D	**EquiScalp™**	Malus Domestica Fruit Cell Culture Extract (and) Ethylhexylglycerin (and) Hydroxyacetophenone	Active ingredient	2.00
	Plantasens® Berto Oxi Complex	Glycerine (and) Water (and) Garcinia Mangostana Peel Extract (and) Guazuma Ulmifolia Leaf Extract (and) Phyllanthus Niruri Extract (and) Chlorphenesin	Antioxidant/ Moisturizer	1.00
	Plantasens® Berto Gotubright	Glycerine (and) Water (and) Centella Asiatica Flower/Leaf/Stem Extract (and) Chlorphenesin	Moisturizer	1.00
	Phenoxetol®	Phenoxyethanol	Preservative	1.00
	Velsan® Flex	Capryloyl/Caproyl Anhydro Methyl Glucamide (and) Water	Solubilizer	0.40
	Perfume	Fragrance	Fragrance	0.50

Procedure

1. In the main beaker, add phase A and stir until the solubilization is complete. Heat to 80°C.
2. Add B while stirring vigorously and heat to 70°C. Maintain this condition (temperature and speed) for 10 minutes.
3. Add phase C to the main beaker and increase temperature to 80°C. Stir vigorously. Maintain this condition for 15 minutes
4. Allow it to cool down.
5. When the temperature reaches 30°C, add phase D, one ingredient after the other, and stir until homogeneous.
6. Adjust the pH if necessary.

Appearance

White yellowish cream / serum

How to use

Dispense 1-2 pumps of the hair nectar into your hands, rub your hands together, and run your hands through your dry or damp hair. Brush your hair for even distribution and dry hair if necessary.

pH / Viscosity

4.0–4.5 / 4000–6000 mPa.s

Stable for 3 months at 2°C, -5°C, 25°C, 45° and under UV exposure.

schülke

Natural Anti-Wrinkle Face Cream | FL-19-0148

Phase	INCI	Trade Name	Supplier	Function	% w/w
A	Cetearyl Glucoside, Cetearyl Alcohol	Emulgade® PL 68/50	BASF	Emulsifier	3.00
	Sodium Stearoyl Glutamate	Eumulgin® SG	BASF	Emulsifier	0.50
	Cetearyl Alcohol	Lanette® O	BASF	Consistency Agent	1.50
	Hydrogenated Vegetable Glycerides	Cutina® HVG	BASF	Consistency Agent	1.50
	Hydrogenated Vegetable Oil	Cegesoft® HF 62	BASF	Emollient	2.00
	Butyrospermum Parkii Butter	Cetiol® SB 45	BASF	Emollient	2.00
	Caprylyl Caprylate/ Caprate	Cetiol® RLF	BASF	Emollient	5.00
	Dicaprylyl Ether	Cetiol® OE	BASF	Emollient	5.00
	Olus Oil	Cetiol® PS 6	BASF	Emollient	3.00
B	Aqua				ad 100
	Glycerin	Glycerol 85%		Humectant	8.00
	Xanthan Gum	Keltrol® CG-SFT	CP Kelco	Stabilizer	0.50
C	Glycerin, Aqua, Khaya Senegalensis Extract, Maltodextrin	Collalift™ 18 BC10014	BASF	Active	1.00
D	**euxyl® ECO 910**		Schülke & Mayr GmbH	Preservative	1.00
	Citric Acid	Citric Acid (50 % aq. solution)		pH-adjuster	q.s.

pH value: 5.0

Procedure:
Heat phase A and phase B separately to 80°C.
Add phase A to B while stirring. Homogenize at 55°C. Add phase C below 40°C and stir.
Add euxyl® ECO 910 and mix until homogeneous. Adjust the pH with citric acid to 5.0.

ROELMI·HPC
Health & Personal Care

Regenerating Serum | C.SK.17.55HPC

Phase	Trade Name	INCI	Supplier	%
A		Aqua		63.60
	CytoFruit® Water Lemon BIO99	Citrus Limon Fruit Extract, Potassium Sorbate	ROELMI HPC	20.00
	Olifeel® Glycerin	Glycerin	ROELMI HPC	2.00
		Xanthan gum		0.20
	PrincipHYAL® Aurora	Sodium Hyaluronate	ROELMI HPC	0.30
		Tetrasodium EDTA		0.10
B	**Olifeel® TD OW**	Polyglyceryl-3 Stearate, Cetearyl Olivate	ROELMI HPC	3.00
	EMotion Light	Tripelargonin	ROELMI HPC	7.00
		Tocopheryl Acetate		0.30
	BiosControl® Synergy ICE	Caprylyl Glycol, Ethylhexylglycerin, o-Cymen-5-ol		1.00
C	**G2White**	Butylene Glycol, Glycyrrhiza Glabra Root Extract	ROELMI HPC	0.30
	Plerasan® Re-Balance	Aqua, Betaglucan	ROELMI HPC	2.00
D		Parfum		0.20
Total				**100.00**

Chemical Physical Parameters:
1. Appearance: LIGHT CREAM
2. Colour: WHITE
3. Smell: LIGHT CHARACTERISTIC
4. pH: 5,5–6,5
5. Density (g/ml): n.a.
6. Viscosity (Fungilab Viscostar-R, s:3, 20 rpm, 25°C): 1000-5000 cP
7. Centrifuge (1 cycle*3000rpm*15'): YES
8. Stability (3 months at 4, 25, 40°C): YES
9. Dropping point (°C): n.a.
10. Surfactant actives content: n.a.

Manufacturing Method:
1. Prepare Phase A under stirring at 70+/-2°C;
2. Prepare Phase B under stirring at 70+/-2°C;
3. Put Phase B into Phase A under mix and mix for 5 minutes;
4. Cool down at room temperature and add Phase C and D.

DISCLAIMER: The formulation above is based on our best knowledge and is provided only as an indication. The end user must verify himself the applicative characteristics, the stability as well as the existence of a patent. The company ROELMI HPC does not give any guarantee and disclaims all responsibility for the use of this formulation. It is intended for operators and technicians only. For more information visit us at www.roelmihpc.com

Givaudan

Givaudan Renames its Flavour and Fragrance Divisions

Taste & Wellbeing and Fragrance & Beauty

Geneva/Switzerland, August 28, 2020. The change aligns with its 2025 strategy: "Committed to Growth, with Purpose" and reflects its expanded product offering.

Givaudan, the world's leading flavours and fragrances company, has announced it is evolving the name of its Flavour and Fragrance divisions to reflect its expanded product offering to its customers.

With immediate effect the Flavour Division will become Taste & Wellbeing, highlighting its global leadership position in flavour and taste, while signalling the expansion of the business into adjacent spaces. The Fragrance Division will become Fragrance & Beauty highlighting its global leadership in Fragrances whilst signalling the expansion into Beauty.

Louie D'Amico, President Taste & Wellbeing said: "Over the past five years, we have expanded our portfolio in a significant way, complementing our industry-leading flavour and taste capabilities with an outstanding offer of natural functional and nutritional solutions. The new name captures this expanded portfolio and the value we bring to customers by going beyond great taste, to create more complete future-facing food experiences."

Maurizio Volpi, President Fragrance & Beauty said: "By reinforcing our leadership in fragrances we want to become the creative partner of choice not only in personal, fabric, hygiene and home care but also in fine fragrance and beauty overall."

The new names reflect Givaudan's purpose of creating for healthier and happier lives, with love for nature, and are aligned with the divisions' 2025 strategic ambitions to shape the future of food, fragrances, and beauty by becoming the innovation and co-creation partner of choice to its customers.

www.givaudan.com

hpci CENTRAL & EASTERN EUROPE
WARSAW | 22 – 23 SEPTEMBER 2021

HPCI CEE 2020 postponed

New Date: 22–23 September 2021

The organizers of the HPCI (Home and Personal Care Ingredients) Central and Eastern Europe regret to inform you that, the HPCI CEE which was scheduled to take place at EXPO XXI Exhibition Center in Warsaw, Poland, from 7 to 8 October 2020, will be postponed to 2021.

Given all relevant circumstances, including the continued unknown variables of the ongoing COVID-19 pandemic, corresponding changes by governments, and challenges to businesses, this was an unavoidable decision.

The HPCI CEE will resume its schedule **22 & 23 September 2021**, at the EXPO XXI Exhibition Centre in Warsaw, Poland.

Whilst it is possible to hold trade shows again in Poland and a comprehensive hygienic concept was developed which would have offered a safe HPCI CEE to all participant, the organizers decided to postpone the show to next year. Due to the still very tense Covid-19 situation and the current travel restrictions for some areas, the organizers have finally decided to cancel the event.

The decision was difficult, as the organizers understand the important role the HPCI CEE serves in offering a platform for new products and technologies, connecting industry professionals, and educating home and personal care professionals.

HPCI CEE organizers will continue to keep exhibitors and visitors informed of further details related to the cancellation.

Specific inquiries may be sent to
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