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Potential Futures,  
my hero 'Mr. Spock'  
and what does this  
have to do with innovation?

## preservation

Alternative and Functional Preservation  
from the Lichen's Secondary Metabolite

**03**

2023

english

## chelating agents

Green Chelating Agents  
for Industrial & Institutional Cleaning

## personal care

A Vitaminizing Boost to Improve Scalp Health

New Approach to Skin Well-ageing

An Instant Lift for Skin  
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Ceramide for Well-ageing

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personal care **6**  
**preservation**  
**Alternative and Functional Preservation**  
**from the Lichen's Secondary Metabolite**



**38** home care  
**chelating agents**  
**Green Chelating Agents for Industrial**  
**& Institutional Cleaning**



column

J. Bode  
**Hello Future – Hello SOFW,**  
**Potential Futures, my hero ‘Mr. Spock’**  
**and what does this have to do with innovation?** **2**

personal care

S. Parisi  
**Alternative and Functional Preservation**  
**from the Lichen's Secondary Metabolite** **6**

A. Perrin, A. Le Mestr, M. Arcioni, I. Garcia, C. Meyrignac,  
 C. Capallere, X. Qu, Y. Jiang, J.M. Botto, A.F. Clay, I. Imbert  
**A Vitaminizing Boost to Improve Scalp Health** **12**

Ò. Expósito, A. Guirado, R. Vallecillo, A. Gallego, M. Mas, P. Riera,  
 D. Luna, S. Laplana, T. Ruiz, S. Ruiz, M. Gibert, K. Lingen  
**New Approach to Skin Well-ageing. Skin Microbiota Rejuvenation** **20**

R. Campiche, F. Pascucci  
**An Instant Lift for Skin through the Power of Algae** **26**

A. Momméja  
**Ceramoides™ HP, a Complex of Plant-based Ceramide for Well-ageing** **32**

home & personal care

B. Hoeltken, M. Zschiesche  
**TENSAGEX Prime – Ether Sulfates with Optimized 1,4 Dioxane Content** **36**

home care

A. Gripp, M. Heus, S.Muresan  
**Green Chelating Agents for Industrial & Institutional Cleaning** **38**

interviews

**Oral Health Care with Jungbunzlauer ERYLITE®** **44**  
**Circular Beauty in Botanical Supply Chains** **46**

formulations

**Index of Advertisers/Imprint** **52**



## Hello Future – Hello SOFW

Potential Futures, my hero 'Mr. Spock' and what does this have to do with innovation?

J. Bode



**Jens Bode** is a passionate Innovator, inspired by experience for work applications.

Employed at Henkel AG until end of 2021 in a hybrid work model as employee and a free-lance innovator. At Henkel he worked at Packaging Design, Market Research & Consumer Insights, International Marketing, Trend-Scout & Innovation Game Changer. Part of diverse Think Tanks he coached the Innovation Philosophy worldwide in summary 24 years of Focus Inspiration, Applied Creativity and Transformation.

Starting as freelancer in 2008, he guided companies in the early stages of their Innovations Phase with his insights and ideas even with first prototypes. Jens loves it to discover the new, promote creative talent in companies and jointly interpret new trends into a concept. Since 2022 he is a 'corporate dropout' and independent innovator.

Jens is married to Nic, architect and expert for healing architecture, both have 2+2 children and they reside in Düsseldorf and Aachen in Germany.

<https://www.linkedin.com/in/der-innologe/>

In my youth **Star Trek** was my favorite show and **Commander Spock**, played by **Leonard Nimoy**, was my hero.

Spock is the Science Officer on board the spaceship Enterprise with the task to logically investigate human behavior. As a half-vulcan he suppresses his emotions and usually reacts with logic and even tempered. A typical saying for Spock is the expression '**fascinating**' which he uses to react to events and phenomenon's which would be unthinkable or threatening. This very expression '**fascinating**' have personally used when I experience things which I find hard to believe. '**Fascinating**' as a short mental break before I start judging as a reflex reaction.

**Fascinating.** What is going on out there? Trends, Scenarios, Wildcards which take us out of our routine on a global level: The Covid-19, Ukraine, Refugee, Raw material, Energy or Inflation Crises and most of all the Management crises. We are seeing companies who are shocked into a paralysis and fear and others who are shook for a little and then are released by an explosive creativity.

Most companies do not use trends as a source for insights which would prepare them for external influences and disruptions. They have their internal processes, work according to standard operations or ISO 56002, the standard for Innovations Management. Standards give us seeming security and security is an illusion. To put it bluntly, it a walking speed compared to the dynamic Enterprise Warp speed.

**Attention Future:** At one of my presentations I heard this comment: '*Jens, I cannot do a thing with your colorful pictures and your provoking thesis for the future, it is too general to me.*'

If you want someone else do the thinking, you have the wrong mind set. There is a need for perception and paying attention to recognize external influences in order to have a strategic foresight as an integral philosophy.







To be exact, it is the identification and analysis of still weak signals which could influence the company on all levels.

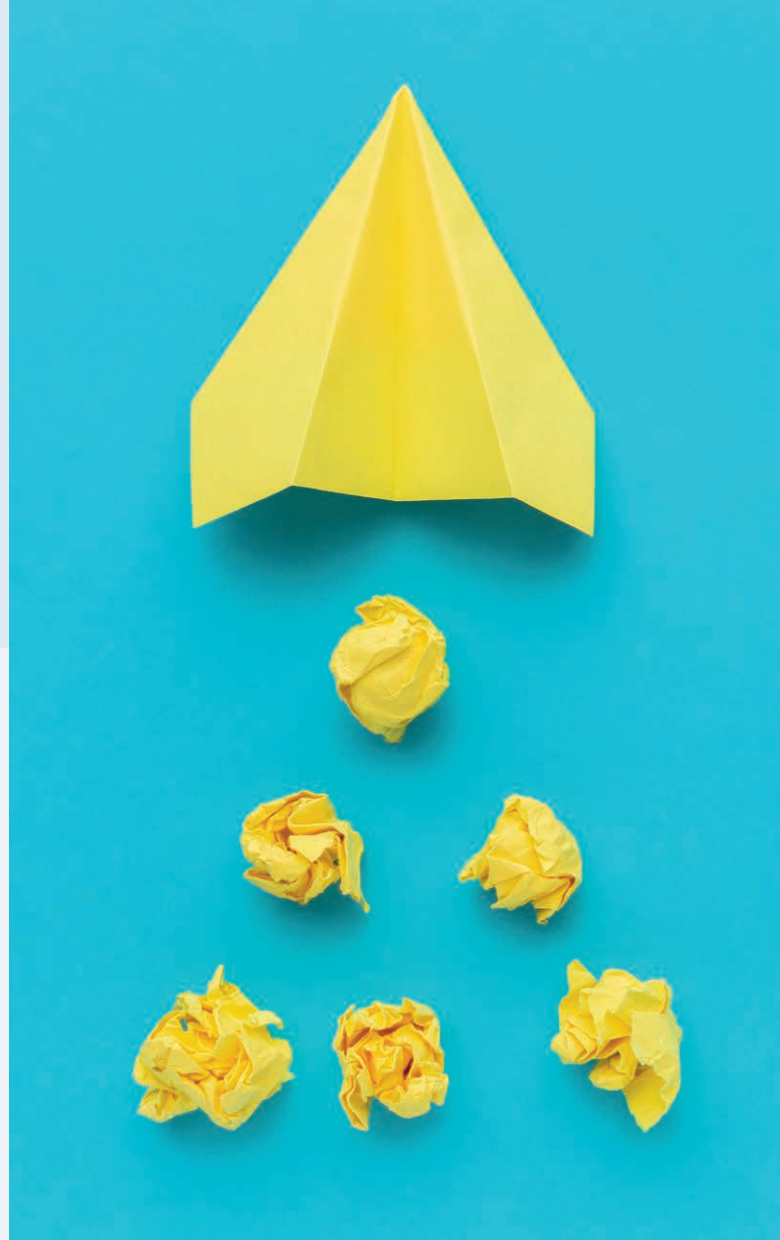
Strategic Foresight is a continual, strategic future oriented process and not an alibi-actionism. Needed is a pro-active approach, as a team effort to work constructively for the future. The basis for this a healthy and alive culture of innovations. To purchase a trend report, save it as an alibi is definitely not enough. A consistent transfer of risk and chance is needed together with a diverse team and not as an one-time event but as a continual, sustainable process.

External influences and trends are challenges like avalanches in slow motion which accelerate unhindered and are only noticed when it is too late and your business model has been hit with full force.

**Self Thinker:** Permanent Change needs courageous thinking of new solutions. We often do not dare to think more courageous and make applications and end up launching the same mini-innovations within the safety containers. We must get away from preservation from mental inactivity and move to active questioning and new options. This means saying goodbye to perfection. Perfection is boring and needed is



- 1. Start now!**
- 2. Try it out** and **trust the process!**
- 3. Development** and **Progress** are better than Perfection!







### ABCD–Mantra (Always Be Connecting the Dots):

Permanent Change needs courageous thinking of new solutions. We often do not dare to think more courageous and make applications and end up launching the same mini-innovations within the safety containers. We must get away from preservation from mental inactivity and move to active questioning and new options. This means saying good-bye to perfection. Perfection is boring and needed is



1. Value **COURAGE:** allow yourself a mind-set outside the norm, think courageously big
2. Value **CURIOSITY:** always try something new and **ATTENTION**, you are allowed to fail.
3. Value **STRENGTH:** you have an individual talent and in synergy with other talents you can set free an unlimited creativity.

**It is OK for Innovation to be fun.** It is really ‘fascinating’ or better frightening how few organizations and politicians are actively involved with analyzing future scenarios and transformations. ‘Anticipate and Lead’: active work with change means to enjoy change and new things, to be prepared and design the future.

**100% commitment:** Invest in an active trend management and tools for applications, invest in an emotional vision and most importantly resources like manpower, time and budget. This is 100 % of an investment in your own company’s future.

**‘The trend is your end  
or the trend is your friend’**

I stick with Commander Spock. Stay curious, be cool in thought and action and be prepared for a fascinating future – your future!



**THE FUTURE  
IS HERE**

### Preview Issue May 2023

My first column with focus on **perception for change** and in my next column we will take a look at the most important trend with consumer goods: **experience economy**.

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# Alternative and Functional Preservation from the Lichen's Secondary Metabolite

S. Paris

## abstract

One of the primary factors driving the shift and growth of the preservative market is the increasing choice for natural cosmetic products, as well as elevated levels of beauty consciousness among consumers. Natural antimicrobial innovations have continued to progress as customers seek alternatives to synthetic preservatives. Furthermore, the growing popularity of natural cosmetic products has driven preservation to become an integral component of the formulation process, focusing not only on quality but also on functionality. Preservation is no longer seen as the final phase in the formulation process, but rather as a need that may operate as multifunctional and offer cosmetic advantages to formulas. Active Micro Technologies has investigated the antimicrobial ability of the lichen *Cladonia rangiferina* as a broad-spectrum protection for cosmetic applications with good anionic system compliance. Featuring antioxidant capabilities, this active will also help protect your skin from internal and external stressors that may bring to premature aging.

## Lichens as a fascinating and inspiring complex life form

Our planet's lichen flora is rich in biodiversity with about 20,000 species. The lichen is neither a plant or an animal. It is essentially a symbiotic relationship between two partners, a fungus and either a green alga or a cyanobacterium (**Figure 1**). For survival, each portion of the lichen is dependent on the other. The fungal component is unable to produce its own nourishment. Its function is to shape the lichen's form and structure. The alga component is there to absorb solar energy and convert it into food for both organisms through photosynthesis. Lichens vary in size from less than 1 mm to over 2m from tree branches while the growth varies in size from hardly noticeable to several millimetres per year.

Lichens are found worldwide and occur in a variety of environmental conditions. They are fascinating because of their capacity to survive in extreme environments; they are one of the most important components of boreal forest vegetation and subarctic ecosystems but they are also able to grow in hot deserts, on the rocks of the alpine peaks or on the rocky coasts swept by the spray.

## Secondary Metabolites in Lichens

The substances that lichens produce to survive in these extreme environments are unique. As an adaptation for life in tough environments, lichens synthesize and accumulate a wide variety of phenolic secondary compounds as a defence against herbivores and to protect against damage by UV-

light in solar radiation. Lichen's secondary metabolites can be a source of energy for soil microorganisms and at the same time, they can inhibit growth of surrounding competitive lichens, mosses, and vascular plants [1]. In most lichen species, those compounds are found in the medulla and may also give some antibiotics and antioxidants properties [2].

Although secondary metabolites are not required for lichen survival and development, studies regarding these compounds have shown several potential benefits. According to published data, the lichens and their secondary metabolites



**Fig 1** Lichens are systems of cooperative organisms and an excellent example of natural symbiosis: they are essentially two separate species living in the same house, despite the fact that they seem as one unified thing.



appear to be good natural antimicrobial agents. Moreover, many lichen extracts have been reported for antioxidant properties due to their phenolic content [3]. Antioxidant agents inhibit and prevent reactive oxygen species, which can cause degenerative diseases. Since now natural antioxidants are preferred over many synthetic antioxidants, this could be seen as an opportunity to incorporate in cosmetic formulation a natural derived antioxidant agent.

### “Reindeer Lichen” as a source of Usnic Acid

Lichens produce more than one thousand secondary metabolites and usnic acid is one of the most abundant. The lichen metabolite usnic acid exhibits various types of biological activity, most notably antimicrobial activity against plant and human pathogens, including inhibitory activity against bacterial strains resistant to antibiotics. Usnic acid is uniquely found in lichens, and is especially abundant in genera, such as *Alectoria*, *Cladonia*, *Usnea*, *Lecanora*, *Ramalina* and *Evernia*.

*Cladonia rangiferina*, also called and known as “reindeer lichen”, is a fruticose that grows in the boreal forests. The name is coming from the fact that it serves as pasture for reindeer, moose, caribou, and musk oxen [4]. Covering large areas, this lichen is able to grow in both hot and cold environments. They often dominate the ground in boreal pine forests and open, low-alpine sites in a wide range of habitats, from humid, open forests to rocks and heat sand, forming an important part of the winter diet of reindeer.

Lichens have been used by humans as food and sources of medicine and dye; *Cladonia rangiferina* has been used for decades for medical purposes since ancient times, treating

colds, arthritis, fever, for example. Many countries have developed commercial pharmacological products based on lichen substances. For instance, usnic acid was used in antiseptic products in Germany [5].

### Antimicrobial activity

The ability of the investigated ingredient, PhytoCide Lichen, to inhibit the growth of a variety of bacteria and fungi was determined using the Minimum Inhibitory Concentration (MIC) test. The results are illustrated, showing that this material provides broad spectrum antimicrobial protection (Figure 2).

The positive MIC screening results warranted further testing to confirm its ability to provide product preservation. 50 g of a generic OW cream formula containing 1% PhytoCide Lichen was weighed into 5 individual containers. Each container was inoculated with one of the 5 test organisms (*E. coli*, *P. aeruginosa*, *S. aureus*, *C. albicans*, and *A. brasiliensis*). The inoculated samples were evaluated at 0, 7, 14, 21, and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining. On the 28th day of testing the samples were re-inoculated and

Organism (ATCC #)	Minimum Inhibitory Concentration (%)
<i>E. coli</i> #8739	0.25
<i>S. aureus</i> #6538	0.12
<i>P. aeruginosa</i> #9027	0.25
<i>C. albicans</i> #10231	0.12
<i>A. brasiliensis</i> #16404	0.12

**Fig.2** Inhibition Activity Data.

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evaluated at 7, 14, 21, and 28 days after the second exposure to determine the number of viable microorganisms (Figure 3).

### Antioxidant activity

An Oxygen Radical Absorbance Capacity (ORAC) assay was conducted to assess the antioxidant capacity of PhytoCide Lichen. The oxygen radical absorbance capacity (ORAC) assay is a standard method used to assess antioxidant capacity of physiological fluids, foods, beverages, and natural products. The assay quantitatively measures a sample's ability to quench free radicals that have the potential to react with and damage cellular components. PhytoCide Lichen was evaluated for its antioxidant capacity. This assay quantitatively measures its ability to quench free radicals that have the potential to react with and damage cellular components.

This assay is based upon the effect of peroxyl radicals generated from the thermal decomposition of 2, 2'-azobis- 2-methyl-propanimidamide dihydrochloride (AAPH) on the signal intensity from the fluorescent probe, fluorescein, in the presence of an oxygen radical absorbing substance. The degree of change is indicative of the amount of radical damage and the presence of antioxidants results in an inhibition in the free radical damage to the fluorescein. The antioxidant protection of the sample can be calculated by comparing it to a set of known standards. Trolox®, a water-soluble vitamin E analogue, with known antioxidant capabilities is used in this ORAC assay as the standard for measuring the antioxidant capacity of unknown substances. ORAC values, expressed in μM of Trolox® equivalents (TE), are calculated using the area under the curves (AUC) of the test product, Trolox®, and the control materials. Trolox® equivalency is used as the benchmark for antioxidant capacity of mixtures since it is difficult to measure individual components.

PhytoCide Lichen exhibited greater antioxidant activity than 200μM Trolox®. The antioxidant capacity of PhytoCide Lichen increased as the concentration increased. As a result, we can assure that its ability to minimize oxidative stress is dose dependent. Maximizing the antioxidant capacity on a cellular level allows for ROS to be dealt with at a rate that provides protection from cellular damage. This cellular damage can

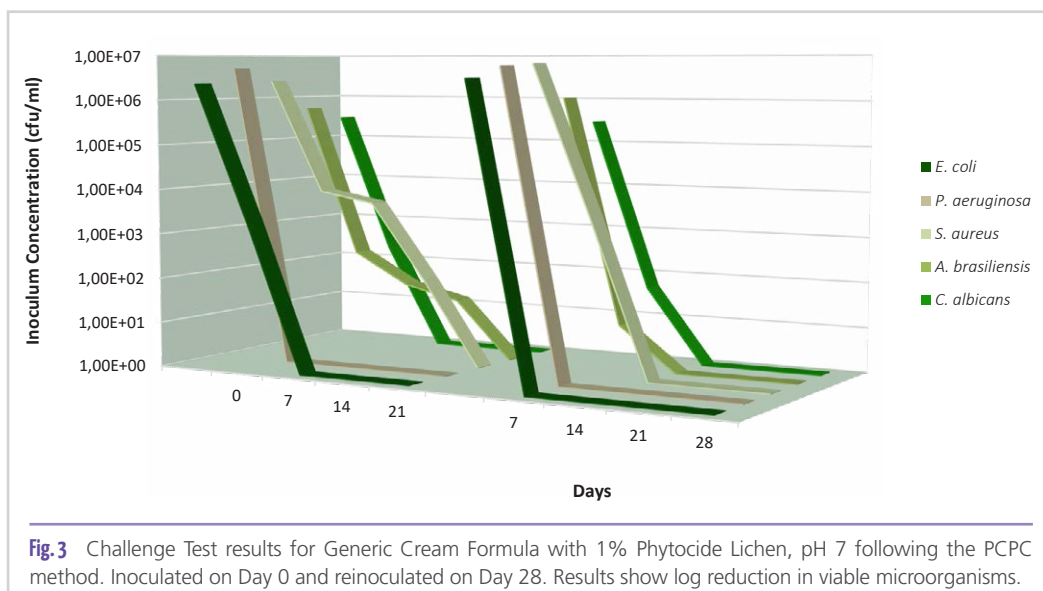


Fig. 3 Challenge Test results for Generic Cream Formula with 1% Phytocide Lichen, pH 7 following the PCPC method. Inoculated on Day 0 and reinoculated on Day 28. Results show log reduction in viable microorganisms.

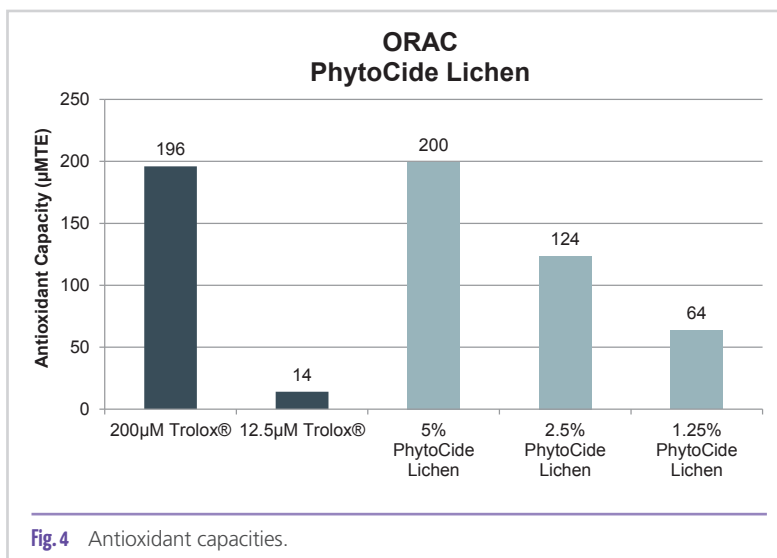


Fig. 4 Antioxidant capacities.

be seen as physical signs of aging such as wrinkles, loss of elasticity, unwanted pigmentation, and skin unevenness with slow regeneration (Figure 4). We can affirm that PhytoCide Lichen is capable of providing antioxidant properties and aids in the anti-aging process through protection at the cellular level.

### Reactive Oxygen Species Scavenging Assay

Low levels of intracellular oxidative stress are produced during normal physiological functions. However, UV irradiation, pollutants, foreign substances, and aging elicit unrestricted increases in reactive oxygen species (ROS). These deregulated augmentations in oxidative stress lead to an acceleration of DNA mutation, cellular senescence, advanced glycation end products, protein oxidation, and collagen degradation. Moreover, when intrinsic antioxidant capacities are reduced, such as during aging, an imbalance between pro and anti-oxidant systems further accentuates these hallmarks of cellular aging.

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Accordingly, a ROS Scavenging Assay was conducted to assess the *in-vitro* effect of PhytoCide Lichen to scavenge unnecessary oxidative stress in dermal fibroblasts. Attenuating excessive ROS preserves cellular homeostasis and blunts intrinsic and extrinsic age-related declines in skin cell function.

Two cell-permeant dyes, CellROX™ Orange Reagent and Hoechst, were utilized in conjunction to provide a specific and quantitative method for determining ROS levels. CellROX™ Orange Reagent fluoresces brightly when bound to ROS indicating oxidative stress, and Hoechst fluoresces when bound to nuclear DNA to indicate cellular nuclei. By displaying the relative fluorescent units (RFU) from the CellROX™ Orange Reagent (ROS Signal) as a function of Hoechst (Nuclear Signal), ROS can be quantified and normalized at the cellular level. To elicit supraphysiological mitochondrial- and non-mitochondrial-derived levels of oxidative stress, the cells were exposed to Antimycin A, a complex III inhibitor of the mitochondrial electron transport chain.

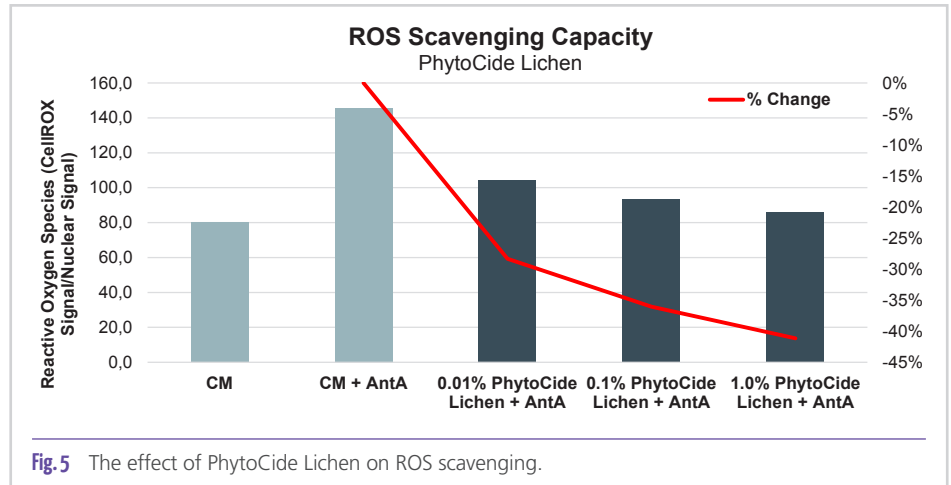


Fig.5 The effect of PhytoCide Lichen on ROS scavenging.

As shown in **Figure 5**, fibroblasts incubated with AntA, a known inducer of oxidative stress, elicited an 82% increase in ROS levels, compared to untreated fibroblasts. These data demonstrate the supraphysiological level of ROS induced by AntA and the magnitude of ROS in fibroblasts is dynamic. Conversely, fibroblasts treated with PhytoCide Lichen at 0.01%, 0.1%, and 1.0% demonstrated 28%, 36%, and 41% reductions in ROS levels compared to fibroblasts treated with AntA, respectively. These data demonstrate PhytoCide Lichen attenuates excessive oxidative stress (**Figure 5**).

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Collectively, intrinsic and extrinsic factors perturb skin homeostasis by stimulating abundant levels of ROS that amplify DNA mutation, cellular senescence, advanced glycation end products, protein oxidation, and collagen degradation. These data indicate PhytoCide Lichen scavenges unnecessary ROS, which may help to attenuate characteristics of cellular aging.

## Summary

Active Micro Technologies harnesses cosmetic functionality, combined with antimicrobial activity to offer a unique story and benefits. Lichen is locally grown in a nursery in the United States for our local manufacturing site based in North Carolina. *Cladonia rangiferina* is maintained in nutrient-rich growth tank and extracted with a unique process to maximize isolation of phenolic secondary metabolites. This process brings to the development of PhytoCide Lichen, a multifunctional active that can help protect the formulation with broad spectrum antimicrobial benefits, give nice antioxidant properties and is easy to handle in most of formulation.

## References:

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# A Vitaminizing Boost to Improve Scalp Health

A. Perrin, A. Le Mestr, M. Arcioni, I. Garcia, C. Meyrignac, C. Capallere, X. Qu, Y. Jiang, J.M. Botto, A.F. Clay, I. Imbert

## abstract

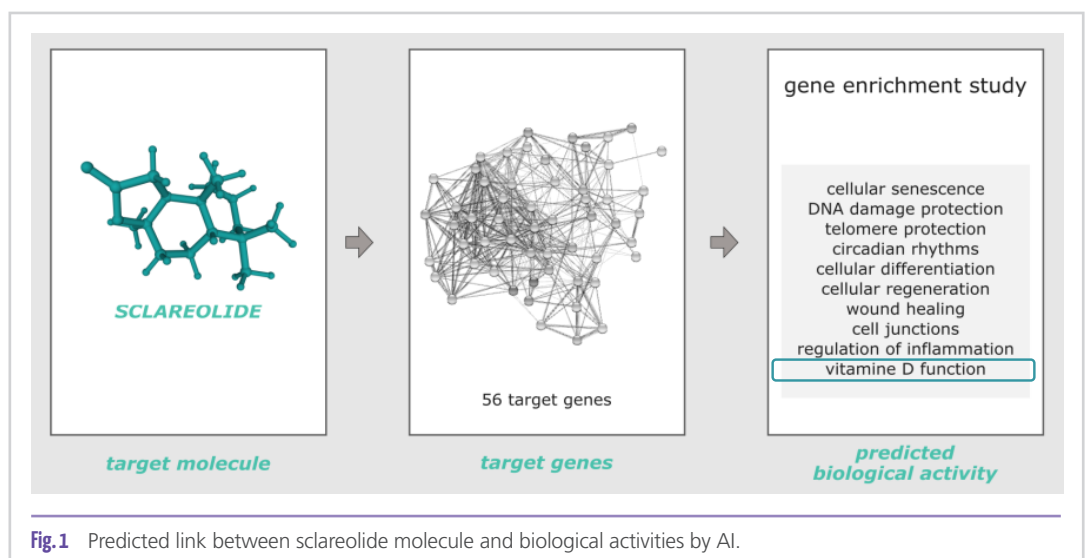
A new botanical extract was developed by Ashland to reduce dandruff formation. It is a natural and biodegradable product which contains sclareolide purified from clary sage flowers, a plant ancestrally used for its purifying and healing properties. A study performed by artificial intelligence pointed out a predictive link between the sclareolide molecule and the vitamin D3, known to play an important role in skin physiology as epidermal differentiation or microbial defenses. Based on these data, this botanical extract was investigated on scalp health improvement in order to decrease dandruff, a widespread uncomfortable scalp skin condition. In a first part, the efficacy of the clary sage extract was studied on yeast cultures, on structural markers of the epidermis and on vitamin D activation. In a second part, a clinical study was conducted on subjects affected by dandruff and treated for 4 weeks with a leave on scalp formulation containing the botanical extract or a placebo. This study demonstrated the benefit of this new product on scalp health by reinforcing the skin barrier function and limiting the appearance of dandruff in association with activation of vitamin D3.

## Introduction

Sclareolide is a purified molecule (INCI name: sclareolide) obtained from clary sage (*Salvia sclarea*). The clary sage is grown in North Carolina, USA, from a unique crop variety obtained after decades-long collaboration with local farmers to select the best variety through plant breeding. Field trials helped to improve crop production techniques: weed management, soil fertility, harvest timing, and to increase the quality of crops. This new botanical extract containing sclareolide is 100% natural, bio-transformed using non-GMO biotechnology and was developed by Ashland to fight dandruff for scalp health and wellness.

Dandruff is a common and chronic scalp problem caused by several abnormalities among them an invasion of characterized fungal strains, a disrupted scalp skin barrier, itchiness and frequently mild-inflammation [1]. *Malassezia restricta*, a yeast naturally present on scalp skin, is one of the main *Malassezia* species involved in dandruff formation [2] demonstrated to be

more abundant in the scalp with dandruff than that in healthy scalp. Their hyper-proliferation induces a change in lipase activity that degrades sebum to give saturated fatty acids and unsaturated fatty acids, that were used to boost their proliferation and disrupt the skin barrier function leading to an abnormal skin permeability [3,4]. The skin barrier disruption is accompanied by an alteration of structural components, like keratins and lipids [1], and corneocyte cohesion leading to a higher desquamation corresponding to the flakes. Itchiness and inflammation are also consequences induced by histamine release and cytokines secretion in the scalp due to skin damage and penetration of pathogens elements in the epidermal structure [1].



Thanks to Artificial Intelligence (AI), sclareolide was predicted to have a link with the vitamin D (**Figure 1**) that plays an important role in skin physiology including barrier formation and antimicrobial defenses. Vitamin D3 is naturally produced by keratinocytes under solar exposure. This synthesis required several enzymes among them CYP27b1 which generate  $1.25(\text{OH})_2\text{D}_3$ , the active form of vitamin D3 [5]. During the barrier formation, vitamin D3 inhibits keratinocytes proliferation and triggers the differentiation process during which specific proteins are expressed to conduct to the formation of differentiated epidermis and formation of the cornified envelope [5,6,7]. Vitamin D3 is also responsible of antimicrobial peptides (AMPs) production to limit the colonization of microorganisms [8,9]. Vitamin D deficiency is known to be associated with chronic inflammatory skin diseases like psoriasis or atopic dermatitis both related to a defect of the skin barrier function, with an abnormal proliferation of the keratinocytes in the basal layer for psoriasis condition and a microorganisms' invasion for atopic dermatitis [7]. Vitamin D deficiency was also demonstrated to be linked to seborrheic dermatitis [10], another skin disease similar to dandruff that both differs by the severity and the location of the skin defect, dandruff being restricted to the scalp.

In the present study, clary sage extract was investigated for its efficacy on dandruff formation in relation with the activation of vitamin D3. The proliferation of the yeast *M. restricta* and its negative effect on epidermal structure were first evaluated. The expression of the enzyme CYP27b1, as well as keratin 10 and lipids as biomarkers of keratinocyte differentiation was then studied on *ex vivo* scalp skin culture. Finally, a clinical study was conducted on subjects affected by dandruff and treated with a hair serum containing clary sage extract at 0.12% or a placebo.

## Materials and methods

### 1. Culture of *Malassezia restricta*

The isolated *Malassezia restricta* strain was obtained from ATCC (ATCC-MYA-4611). *M. restricta* was grown in modified Leeman-Notman (mLN) agar media (1% [w/v] Peptone; 1% [w/v] glucose; 0.2% [w/v] yeast extract; 0.8% [w/v] Ox bile salt; 0.05% [w/v] glycerol monostearate; 0.5% [v/v] Tween® 60; 2% olive oil; 1% [v/v] glycerol; 1.5% [w/v] agar and 1.0L water; and cultured at 34° for 5-7 days.

### 2. MIC determination

*M. restricta* was routinely grown on modified Leeman-Notman (mLN) agar. The broth microdilution assay was performed following EUCAST guidelines (www.eucast.org) with slight modifications. Between five and ten colonies were suspended in a modified RPMI 1640 media supplemented with resazurin at a O.D. (Optical Density) of 0.1. Serial dilutions of clary sage extract (0.0005% to 0.12%), zinc pyrithione (8% – 0.0313 µg/mL) and ketoconazole (0.25 – 0.0010 µg/ml) were prepared in DMSO then in modified RPMI 1640 and dis-

tributed in triplicate for clary sage extract and in sixuplicate for zinc pyrithione and ketoconazole in a 96-well plate. An equal volume of *M. restricta* inoculum was added to the test samples at different concentrations. Following 60h of incubation at 34°C, resazurin fluorescence was analyzed at 530 nm and 590 nm.

### 3. Scalp skin culture

Normal human scalp skin samples were obtained from plastic surgery intervention of female donors. After removal of subcutaneous fat, skin biopsies were cut with a 6 mm diameter punch and were cultivated on culture medium containing 50% of DMEM 1 g/L glucose (Lonza) and 50% of Ham's-F12 (Lonza) supplemented with 10% of FBS (Lonza), 2 mM of L-glutamine (Lonza) and 100 µg/ml of Primocin\* (InvivoGen). Skin biopsies were maintained at 37°C in a humidified atmosphere containing 5% of CO<sub>2</sub>. They were treated topically once a day with 0.12% of clary sage extract diluted in placebo (Ceraphyl 368) or with the placebo for 48 hours. Biopsies were then fixed in formaldehyde and embedded in paraffin. Sections of 4 µm thickness were cut with a microtome (Shandon) and collected on glass slides (Thermo Scientific).

### 4. sRHE culture

For scalp reconstructed human epidermis (sRHE), keratinocytes were extracted from scalp skin samples of female donor. Cells were seeded on inert polycarbonate membrane and were air-lifted for 10 days on a chemically defined medium at 37°C in a humidified atmosphere containing 5% of CO<sub>2</sub>.

The tissues were stressed with application of *M. restricta* (OD= 2.8) for 48 hours and then were treated topically once a day with 0.12% clary sage extract diluted in placebo (ceraphyl 368) or with the placebo for 48 hours again.

### 5. Fluorescent stainings

Sections were deparaffinized and rehydrated. For immunostainings, heat-mediated antigen retrieval was performed using EDTA (Sigma) buffer 1.3 mM pH 9 for CYP27b1 or citrate (Sigma) buffer 0.01 M pH 6 for keratin 10. After saturation of unspecific sites with 5% BSA (Bovine Serum Albumin, Sigma) for 30 minutes, CYP27b1 or keratin 10 primary antibodies (1/1000, rabbit monoclonal, Abcam) were applied for 2 hours. The secondary antibody anti-rabbit Alexa Fluor® 488 (1/1000, Invitrogen) was then applied for 1 hour. For Nile red staining, a solution of Nile Red (Sigma) at 100 nM was added for 10 minutes. Finally, sections were mounted with Fluoromount-G\* containing DAPI (4',6'-diamidino-2-phenylindole) fluorescent dye (Invitrogen).

### 6. Lucifer yellow staining

At day 14, 1 mM of lucifer yellow fluorescent dye was added topically on sRHE for 2 hours. sRHE were then washed and removed from their insert. They were fixed in buffered 10% formalin and embedded in paraffin. Sections of 4-µm



thickness were deparaffinized, rehydrated and mounted in Aquatex\* (Merck).

## 7. Image acquisition and statistical analyses

Pictures were acquired with a Nikon Eclipse Ni-E microscope equipped with a DS-Fi3 Nikon camera, using NiS-AR (Nikon) acquisition software. Six photos per condition were analyzed with Volocity\* image analysis software (Improvision). Statistical analyses were performed using Student's *t*-test for independent samples with one-tailed direction of rejection.  $p \leq 0.05$  were considered as significant,  $p \leq 0.01$  as very and  $p \leq 0.005$  as highly significant.

## 8. Dosage cytokine

After sRHE culture, media were collected and an ELISA (Enzyme-Linked Immuno Sorbent Assay) dosage was performed to quantified interleukin 8 (Duo Set kit DY208-05, R&D system) according to provider's specifications.

## 9. Clinical evaluation

10 human subjects (18-65 years old, 2 males, 8 females) suffer from scalp dandruff or claim having a sensitive scalp skin (screened with 35 questionnaire) were selected to participate in this study. 5ml of the hair serum with/without 0.12% clary sage extract were applied on each half scalp (active versus placebo) once per day for 4 weeks. The adherent scalp flaking score (ASFS) was evaluated by expert grading on dandruff, the skin barrier function was evaluated by TEWL measurement by Tewameter (Tewameter® TM 300, CK) and the Dermoscopy imaging was taken by CBS (CBS-908). The measurements were double-blind, randomized, split-scalp and carried out every 2 weeks. The clinical studies were performed in accordance with the Declaration of Helsinki and passed the toxicological and safety evaluations. The statistical difference of data obtained from clinical evaluation was analyzed with one-tailed, the paired sample *t*-test or Wilcoxon Signed Rank test, depending on whether the data followed a normal distribution.

## Results

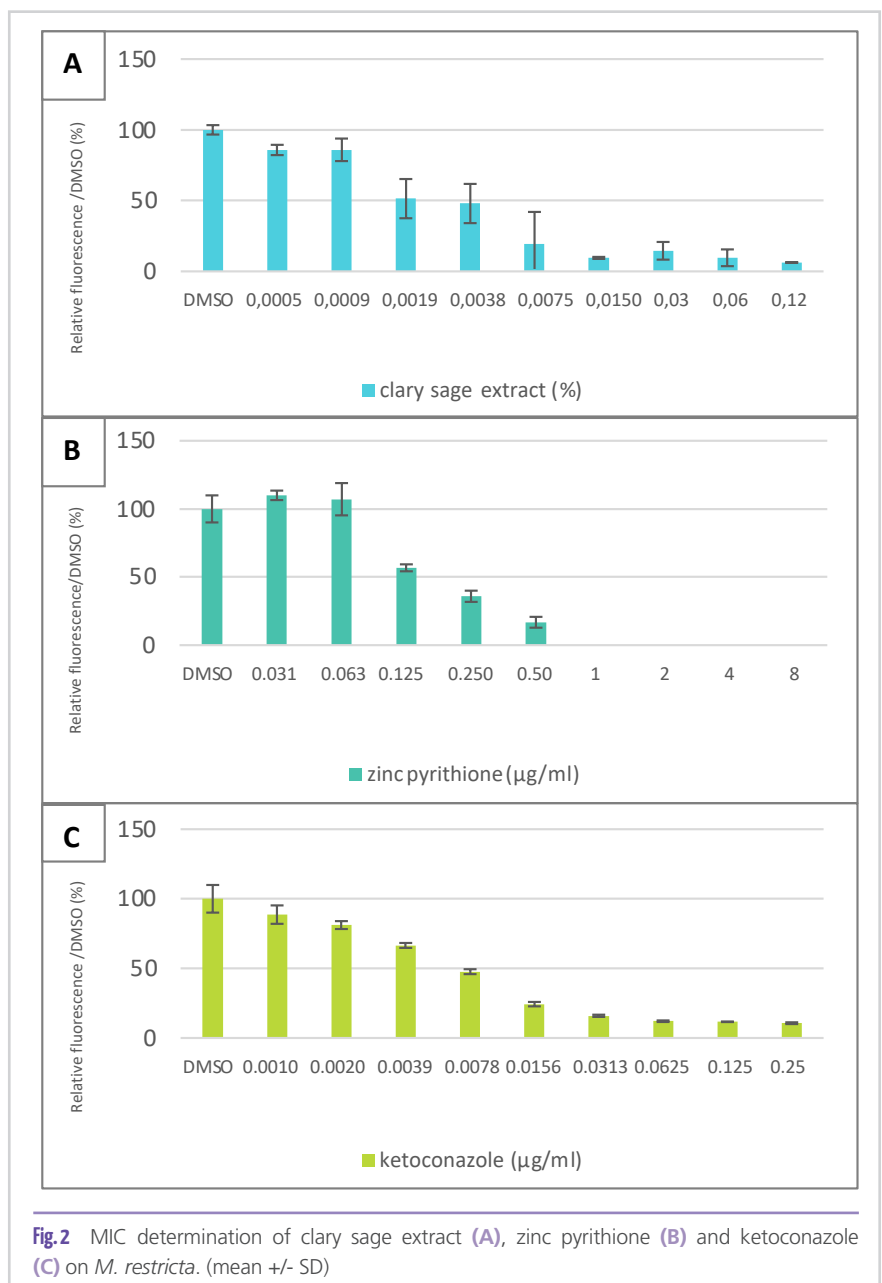
### Inhibitory effect on yeast growth

The anti-microbial effect of clary sage extract was studied on *M. restricta* culture, one of the main yeasts involved in dandruff condition. The MIC (Minimum inhibitory concentration) for the extract on

*M. restricta* growth was evaluated by fluorescence intensity based on EUCAST antifungal MIC method and was compared to those of ketoconazole and zinc pyrithione, 2 well-known anti-fungal compounds frequently used in commercial products to fight dandruff. The products were tested for an incubation of 60h with yeast for a range of concentrations surrounding those normally used. The results showed an efficacy of clary sage extract on inhibition of *M. restricta* growth with a MIC at 0.015%. This inhibitory effect was relatively close than those obtained with the 2 others chemical compounds, zinc pyrithione and ketoconazole (Figure 2).

### Vitamin D activation

As vitamin D function was highlighted by the AI study as target to sclareolide, CYP27b1, the enzyme responsible of the activation of vitamin D, was studied. An application of 0.12% of the botanical extract on *ex vivo* scalp skin for 48 hours was associated with an increased level of this enzyme





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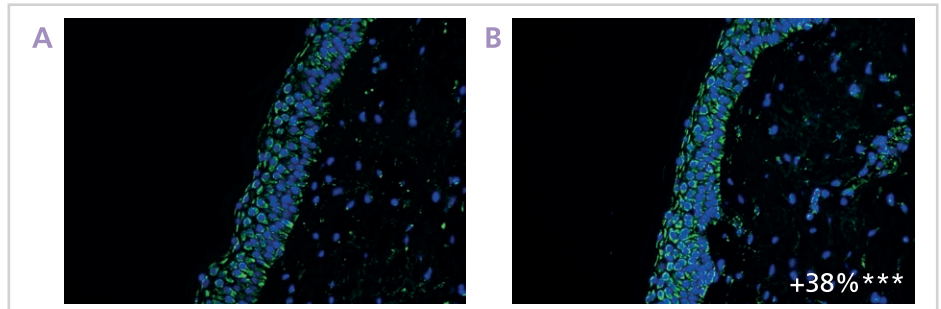
CYP27b1 suggesting a higher amount of cellular vitamin D available in its biologically active form (Figure 3).

**Scalp barrier reinforcement**

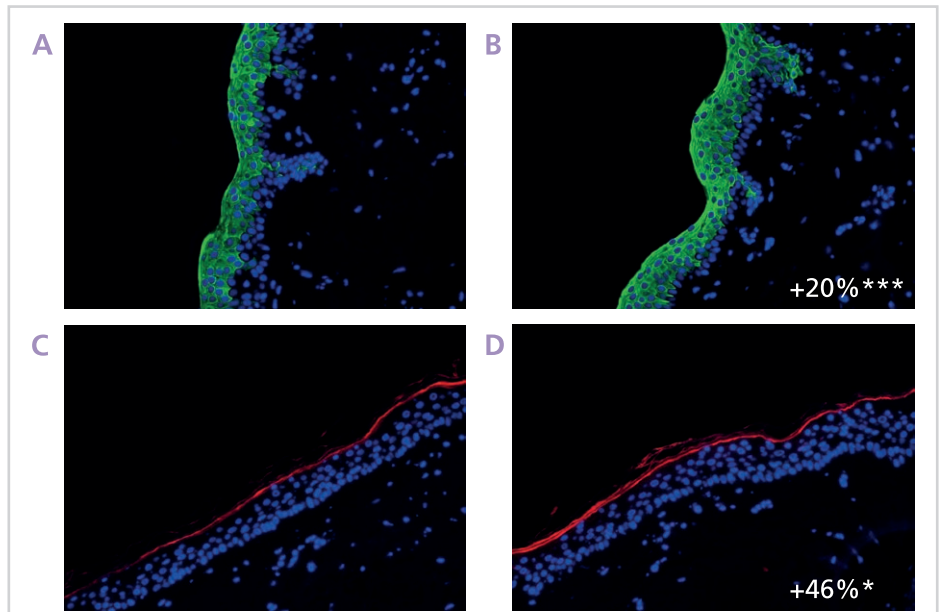
Scalp skin barrier is defective in individual affected by dandruff. Keratin 10 and lipids are fundamental components of the differentiated layers of epidermis and play an important role in skin structure and skin permeability. Their expressions were evaluated to test the efficacy of the extract on barrier function. The results showed that the synthesis of keratin 10 and total lipids were increased in scalp skin after a 48h-treatment with 0.12% of the biofunctional (Figure 4).

**Scalp barrier protection**

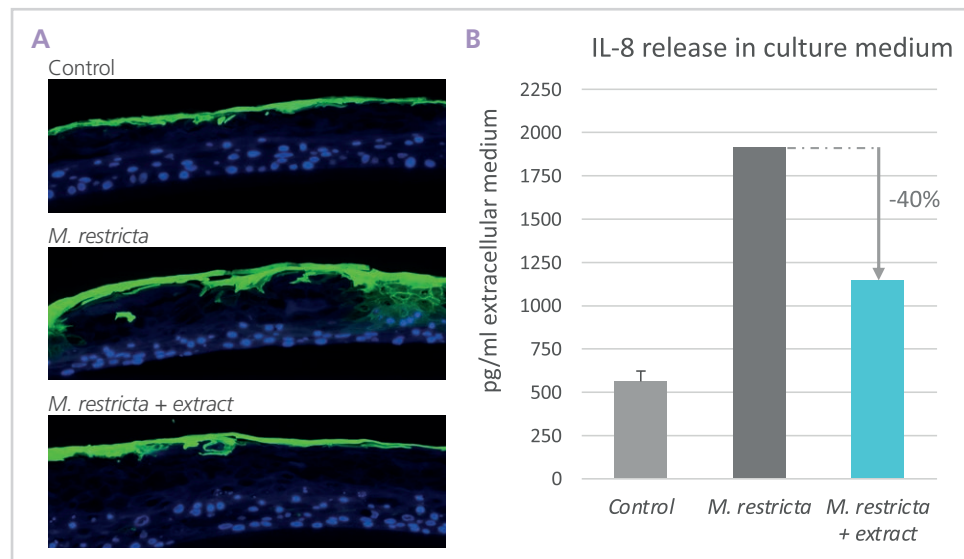
During dandruff formation, scalp epidermis is submitted to yeast invasion that weaken the structure and lead to pathogen penetration and skin inflammation. The topical application of the fluorescent dye lucifer yellow on scalp reconstructed human epidermis (sRHE) infected with *M. restricta* showed a higher product penetration in epidermis as seen by green fluorescence due to damage in the skin barrier induced by the yeast as well as a higher re-



**Fig.3** Immunodetection of CYP27b1 (in green) on ex vivo scalp skin biopsy treated with placebo (A) or 0.12% clary sage extract (B). DAPI blue nuclear counterstain. 20x objective lens. Statistical analyses were expressed versus placebo (mean +/- sem; \*\*\*: highly significant with Student's t-test).



**Fig.4** Immunodetection of keratin 10 in green (A,B) and Nile red fluorescent detection of total lipids in red (C,D) on ex vivo scalp skin biopsy treated with placebo (A,C) or 0.12% clary sage extract (B,D). DAPI blue nuclear counterstain. 20x objective lens. Statistical analyses were expressed versus placebo (mean +/- sem; \*\*\*: highly significant, \*: significant with Student's t-test)

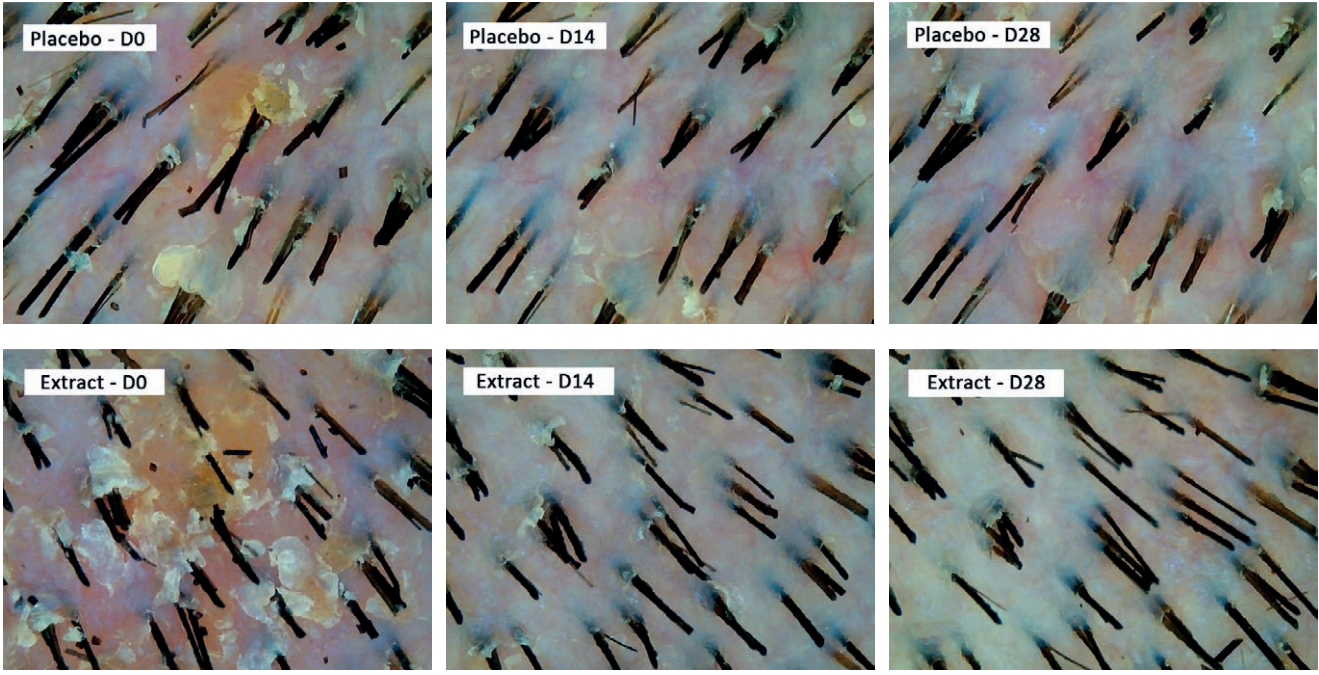


**Fig.5** (A) Evaluation of skin barrier integrity by studying the penetration of a fluorescent dye (green) in sRHE control or infected with *M. restricta* for 48h and treated with placebo or 0.12% clary sage extract for next 48h. DAPI blue nuclear counterstain. 20x objective lens. (B) IL-8 measurement in culture medium of sRHE infected with *M. restricta* for 48h and treated with placebo or 0.12% clary sage extract for next 48h.

lease of interleukin-8 (IL-8), a pro-inflammatory cytokine, in the culture medium. A treatment for 48 hours with 0.12% clary sage extract on sRHE infected with *M. restricta* prevented the penetration of the fluorescent dye in the epidermis structure showing a skin barrier protection by the biofunctional and a mitigated release of IL-8 (Figure 5).

**Dandruff improvement**

To prove *in vivo* efficacy of the botanical extract, a clinical study was conducted on subjects with dandruff treated for 28 days on one half scalp with the biofunctional formulated at 0.12% in a hair serum and on the other half



**Fig. 6** Pictures of scalp with dermoscopy imaging of a subject (#007, female, age 37) treated both with 0.12% clary sage extract on one side and with placebo on the other side for 28 days.

scalp with a placebo formulation. The application of formulation containing clary sage extract at 0.12% was associated

with visible decrease of flaking and erythema compared to placebo application (**Figure 6**).

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The evaluation of flaking by adherent scalp flaking score (ASFS) showed both the placebo and the extract side had less adherent dandruff at D14 and D28, but the difference of ASFS level (D28-D0) was significantly increased by 200% for the extract side with more than 70% volunteers improved, as compared to placebo side ( $p < 0.05$ ). The data showed that there is an improvement of scalp desquamation with hair serum containing 0.12% clary sage extract (Figure 7).

Scalp barrier function was evaluated clinically by transepidermal water loss (TEWL) measurement. The data showed that the scalp water loss was significantly reduced for both the placebo and the extract side at D14 and D28, but the difference of TEWL level was improved on the extract side compared to placebo on more than 90% volunteers at D14 and more than 70% volunteers at D28. The data showed an improvement of skin barrier function with hair serum containing 0.12% clary sage extract (Figure 8).

### Discussion and Conclusion

The clary sage extract, containing sclareolide, is a naturally and biodegradable new product developed by Ashland. It is designated to prevent dandruff development by targeting 2 related causes: the yeast colonization and the skin barrier integrity implying vitamin D activation.

The efficacy of clary sage extract was demonstrated to inhibit proliferation of *M. restricta*, one of the main yeast species involved in dandruff development, with the same efficacy than 2 products used in hair care formulation to fight dandruff, zinc pyrithione, that will be forbidden for its potential effect as endocrine disruptor, and ketoconazole, used as therapeutic agent for seborrheic dermatitis, a more serious form of dandruff.

Clary sage extract application on scalp skin biopsies was associated with a stimulation of CYP27b1 expression, the enzyme

responsible of vitamin D3 activation, suggesting a higher content of active vitamin D3 in keratinocytes. This result was accompanied by a stimulation of keratin 10 and lipids synthesis, 2 components involved in barrier formation suggesting a reinforcement of this function.

The implication of clary sage extract in scalp skin protection was established using scalp reconstructed human epidermis infected with *M. restricta*. A restoration of the skin barrier function associated with a decrease of inflammation on sRHE

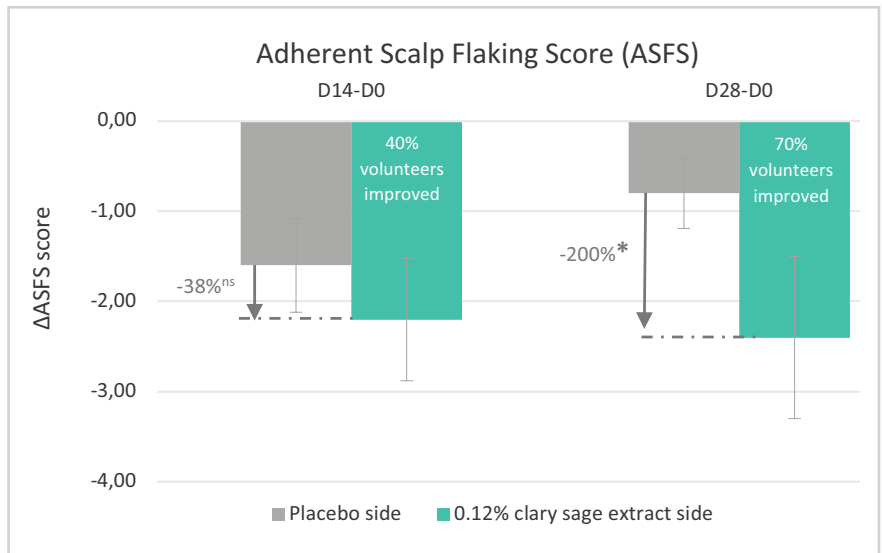


Fig.7 Determination of the difference of adherent scalp flaking score ( $\Delta$ ASFS) on subjects treated both with 0.12% clary sage extract on one side and with placebo on the other side for 14 and 28 days (n=10, ns: not significant,  $p > 0.10$ , \*: significant,  $p < 0.05$ )

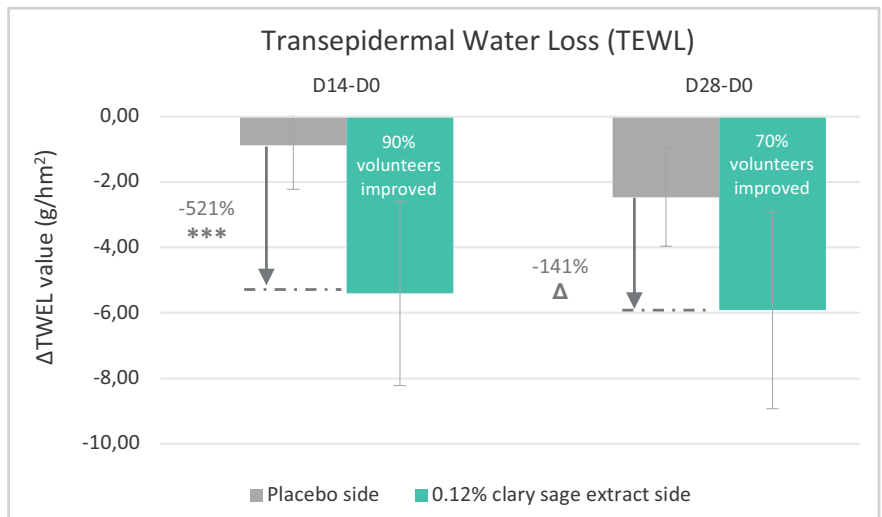


Fig.8 Difference of TEWL measurement ( $\Delta$ TEWL) on subjects treated both with 0.12% clary sage extract on one side and with placebo on the other side for 14 and 28 days (n=10; Δ: directional,  $0.05 < p < 0.1$ ; \*\*\*: highly significant,  $p < 0.005$ )

was observed as seen by a reduction of the penetration of the fluorescent dye in the epidermis depth and a decrease in IL-8 release in the culture medium.

Finally, these results were confirmed by a clinical study conducted on subjects affected with dandruff and treated with a hair formulation containing clary sage extract or not. After 4 weeks of treatment with the extract, a reduced level of flaking and redness as well as a better scalp barrier function were observed compared to placebo translating an improvement of dandruff condition.

To conclude, clary sage extract was proven to have a great potential in hair care formulation as it was shown to limit the appearance of dandruff and restore a healthy scalp barrier in connection with a vitamin D3 activation.

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# New Approach to Skin Well-aging. Skin Microbiota Rejuvenation

Ò. Expósito, A. Guirado, R. Vallecillo, A. Gallego, M. Mas, P. Riera, D. Luna, S. Laplana, T. Ruiz, S. Ruiz, M. Gibert, K. Lingen



With age, the skin microbiome changes and evolves from producing a postbiotic of youth markers (Youth Biome Markers, YBMs) to secreting a postbiotic of ageing markers (Senile Biome Markers, SBMs). These SBMs are activated by Quorum Sensing, a microbial communication system which allows them to coordinate their behaviour, turning them more virulent and producing degradative enzymes. These enzymes are harmful for the skin and lead to the appearance of signs of ageing: inflammation, redness, pores, loss of firmness and appearance of wrinkles.

The active Quora Noni™ *biomics*, obtained from *Morinda citrifolia* (noni) stem cells, inhibits the Quorum Sensing. By doing so, it rejuvenates the skin microbiota, reducing the SBMs and therefore, the wrinkles. At the same time, it increases the skin firmness and improves the imperfections (redness and pores) on mature skin profiles.

cence, oxidative stress, excessive pro-inflammatory signals [8]), and it responds to an adaptative mechanism of survival.

The skin microbiota ageing takes place due to two accumulative changes which occur with time [9]:

- Initially, changes in gene expression, mutations and gene transfers to adapt to the local environment: for example, activating lipases with the aim of obtaining nutrients, or forming biofilms to enhance their adherence to the surface and compete with other microorganisms. These changes modify microbial metabolism and signaling pathways, becoming more aggressive for the skin where they live.
- In the long run, the substitution of species which alters the microbiome composition: the so-called Age-Related Microbial Shifts (ARMS). First, a reduction of the diversity unbalances the microbial ecosystem. This allows the appearance of new dominant strains, and the colonization of opportunistic pathogenic species. In this process, the new species proliferate excessively and end up increasing the microbial diversity, but this diversity increase is harmful for the skin, as it is the pathogens what increases.

As a consequence, at mid and long-terms there is a progressive increase of the SBMs, which accelerate the skin ageing. This microbiota ageing has a harmful effect on the skin, due to the virulence factors involved in the process.

## Introduction

### Hallmarks of ageing and the role of skin microbiota

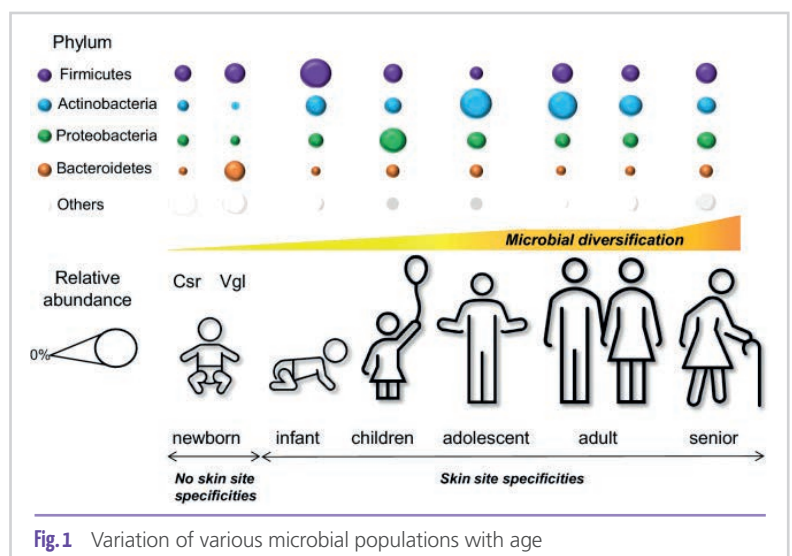
Traditionally, the hallmarks of ageing included a variety of aspects from the telomere shortening, the oxidative stress and the cellular senescence until epigenetics, among others [1]. Nowadays, we know that the skin microbiota plays a key role in cutaneous homeostasis, and the skin microbial ecosystem evolves with age [2,3,4].

### Does the skin microbiota also age?

The cutaneous microbiota evolves as we age, so the skin microbial profiles can predict our age with a margin error precision of 4 years [5] (Figure 1).

Thus, the skin microbiota ages, and the characteristic trait of this process is that its behaviour changes to virulence [6,7]. This change with its relationship with the host affects the ageing, including the skin. The resulting microbial postbiotic is rich in Senile Biome Markers (SBMs).

The microbiota dysbiosis related to ageing is caused by external and internal factors (cellular senes-



### Quorum Sensing, SBMs and skin ageing

The Quorum Sensing is a microbial communication system which allows bacteria to count themselves, and, when a population threshold is surpassed (quorum in Latin), coordinate a behaviour. This turns them virulent and makes them proliferate excessively and form biofilms. This produces an imbalanced cutaneous microbiota which irritates and harms the skin.

The **Senile Biome Markers (SBM)** are microbial factors which favor the growth and survival of microorganisms in a pathogenic scenario. These factors are activated by Quorum Sensing and include a wide range of components to potentiate the adhesion (biofilm formation), resist the immune system and harm the tissues to obtain nutrients for their growth [10].

Among the **microbial molecules which activate the Quorum Sensing**, we find the Homoserine Lactones (HSL) [11], while within the **most relevant SBMs** we need to highlight the **microbial degradative enzymes**, such as **proteases, phosphatases and lipases**. These SBM are secreted outside the microbial cells, affecting the key structural components of skin.

In optimal conditions, i.e., in a young biome, the profile of **Free Fatty Acids (FFAs)** produced by the commensal microor-

ganisms has a key role in maintaining the host's youth (**Youth Biome Markers, YBMs**): they inhibit the Quorum Sensing. Among the **FFAs which inhibit the Quorum Sensing**, **octanoic acid, or caprylic acid**, stands out [12].

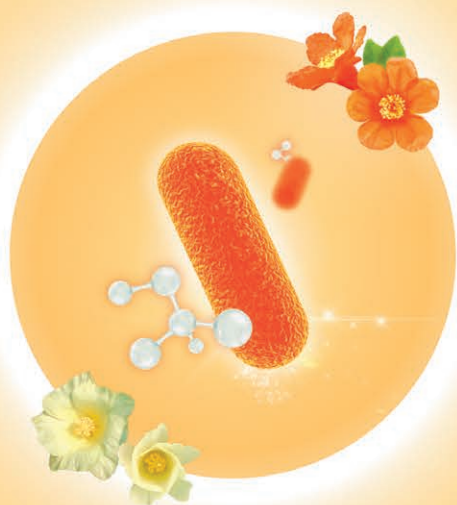
Furthermore, when the cutaneous microbiota produces SBMs, the skin loses its capacity to face microbial aggressions. Some of the key components needed by the skin to defend itself are the **Defensins, Antimicrobial Peptides (AMPs)** [13].

Therefore, **skin microbiota activates the Quorum Sensing and produces SBMs when ageing**:

- The Homoserine Lactones (molecules of Quorum Sensing) increase
- The octanoic acid (inhibits Quorum Sensing) decreases
- The Defensins (Antimicrobial Peptides) decrease

And as a result, the inflammation increases (more bacterial virulence), as well as the oxidative stress and the cellular senescence. At the same time, the skin barrier function and the collagen synthesis decrease. This favors some **signs of ageing** such as **dryness, redness, pores, loss of firmness and appearance of wrinkles**.

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## Reversing the skin microbiota ageing

Quora Noni™ *biomixs* is a 100% natural active ingredient from noni (*Morinda citrifolia*) stem cells. This traditional Polynesian plant is extremophile and capable of colonizing the basaltic rock from the volcanic islands of the Pacific. To achieve that, it has developed the ability of inhibiting the Quorum Sensing from surrounding microorganisms very efficiently.

Vytrus has developed a cocktail which inhibits the Quorum Sensing thanks to the activity of Noni stem cells. The product has a unique composition: a mixture of terpenes, polyphenols and phyto-sterols capable of rejuvenating the cutaneous microbiota and the skin at the same time.

## Biological activity

### *In vitro*

#### Anti-biofilm effect (Quorum Sensing inhibition)

The biofilm formation was studied in various microorganisms, each cultured in its specific culture medium. The growth at 24h from initial inoculums were compared, in suspension and in discs of borosilicate glass where the biofilms can be formed, in absence or presence of the active. Apart from the CFU count in suspension and on the discs, on the latter, photographs were taken using Laser Scanning Confocal Microscopy. The biofilm formation inhibition was analyzed in *Cutibacterium acnes* and in *Staphylococcus aureus* (Figure 2).

In all studied bacteria, 10% Quora Noni™ *biomixs* inhibited the biofilm formation by 99% without having a bactericidal effect, i.e., maintaining the bacterial cells alive.

#### Reduction of lipase activity on *S. aureus* (inhibition of SBM)

By fluorescence, the lipase activity was measured (Relative Fluorescence Units, RFU) at 48 h of a standard lipase and of the supernatant of a *S. aureus* culture in absence or presence of Quora Noni™ *biomixs*. The active inhibited the lipase activity by 98.9%, reducing the production of SBM.

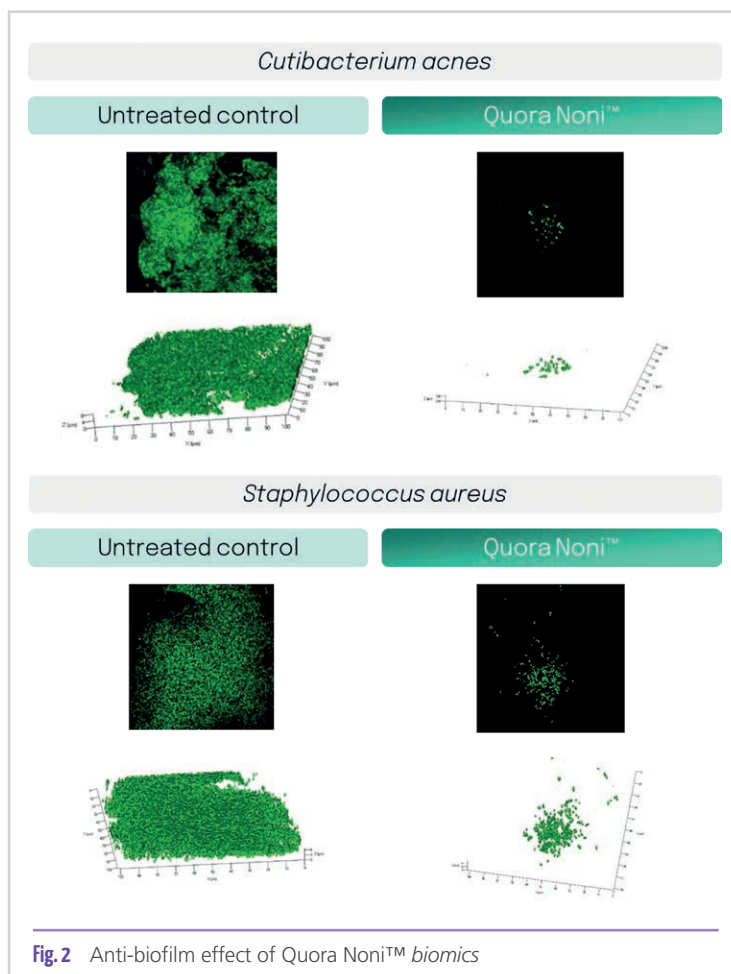


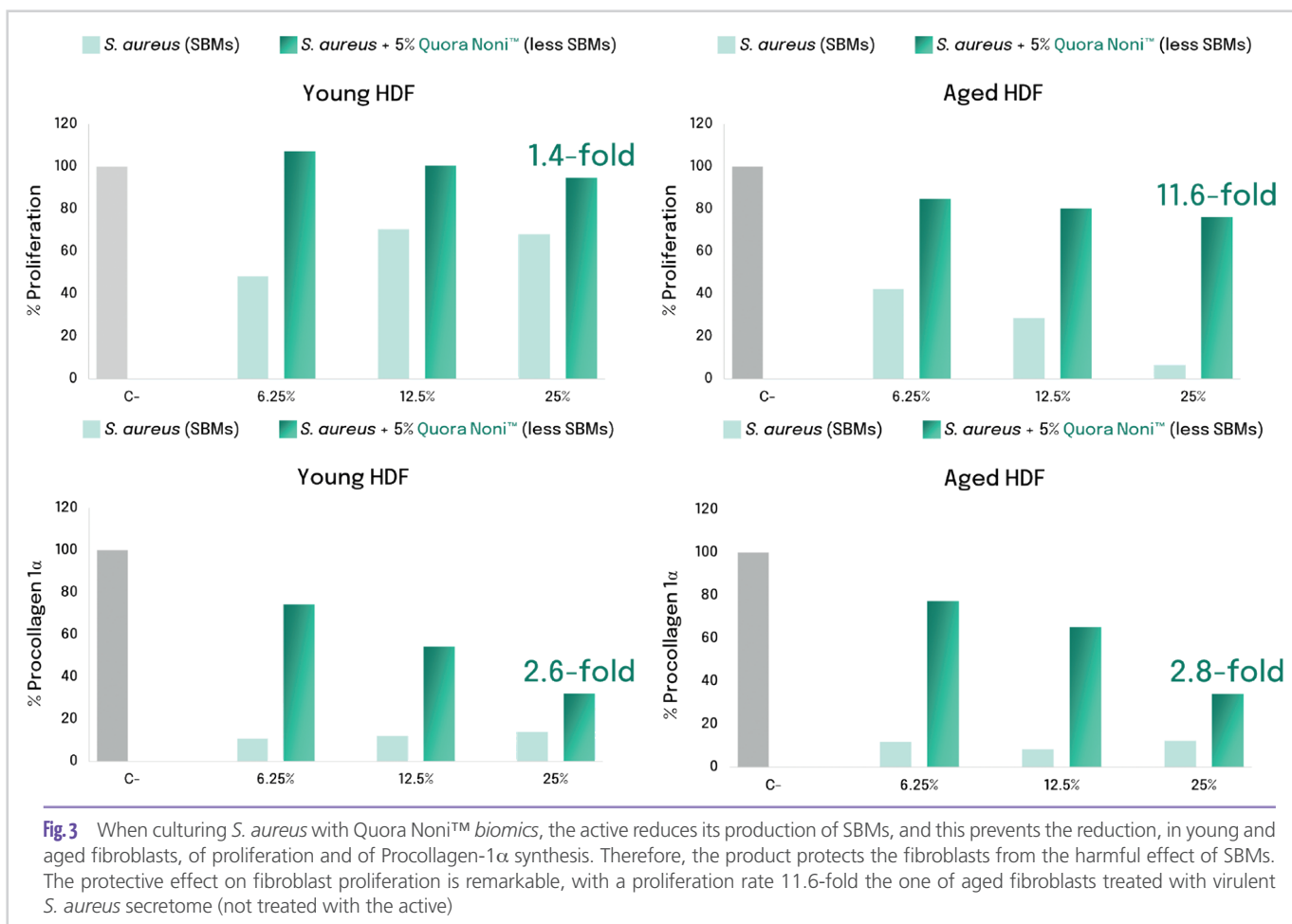
Fig. 2 Anti-biofilm effect of Quora Noni™ *biomixs*

#### Effect of the SBMs on fibroblasts and protection with the active

Vytrus has carried out, for the first time, research on the effects of a senile microbiota on skin cells. Human Dermal Fibroblasts (HDF) were used, both young (with only 2-6 subcultures) and aged (replicative senescence with 10-12 subcultures), and the proliferation capacity as well as the Procollagen-1 $\alpha$  synthesis were analyzed.

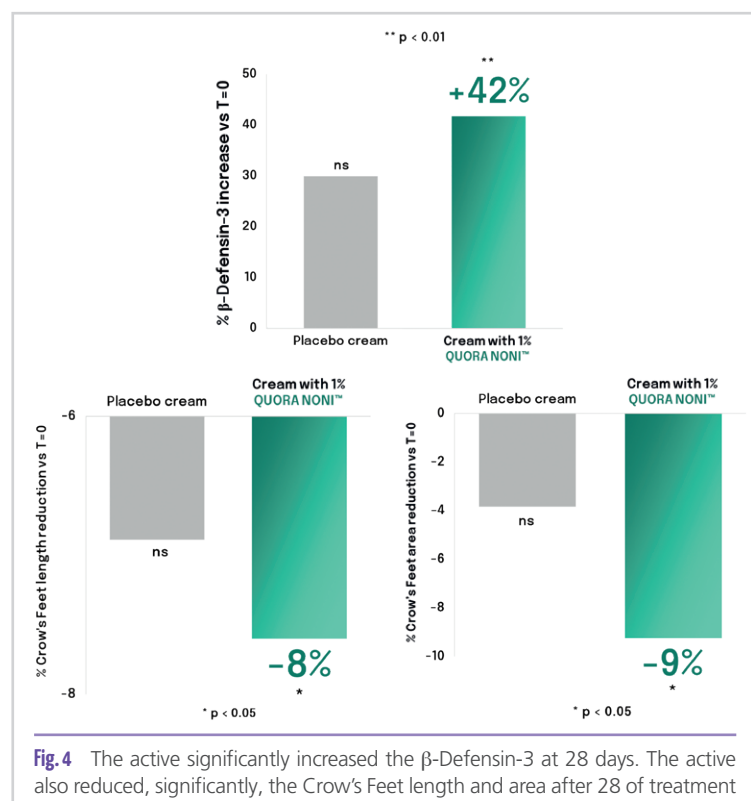
For the study, a model of Senile Biome Markers (SBMs) was designed and prepared, based on a cocktail of microbial degradative enzymes (phosphatases, proteases and lipases), from potential pathogens from the skin microbiota: *Staphylococcus aureus* and *Candida rugosa*. The selected enzymes were the Protein A-alkaline phosphatase and the V-8 protease of *Staphylococcus aureus*, and lipase from *Candida rugosa*.

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The cocktail of microbial degradative enzymes remarkably reduced the proliferation and the Procollagen-1 $\alpha$  synthesis on both HDF, young and aged.

After that, the effect of the virulent secretome from *S. aureus* (rich in SBM) was measured after 72 h of incubation, on young and aged HDF. In parallel, *S. aureus* was cultured for 72 h with a 5% Quora Noni™ biomics, and the resulting microbial secretome was applied on young and aged HDF (Figure 3).



### Clinical evaluation

#### In vivo 1:

Carried out with 20 volunteers between 40 and 60 years old, double blind and placebo-controlled (hemi-facial applications), at the dosage of 1% of the active, with two daily applications for 28 days.

For the first time *in vivo*, the levels of  $\beta$ -Defensin-3 (an antimicrobial peptide, AMP) were measured at the beginning and at the end of the treatment.

Also, the length and the area of the Crow's Feet were measured at day 0 and at day 28 (Figure 4).

The ingredient significantly increased the  $\beta$ -Defensin-3 production by 42% after 28 days of treatment, thus reinforcing the skin's defenses. The active also

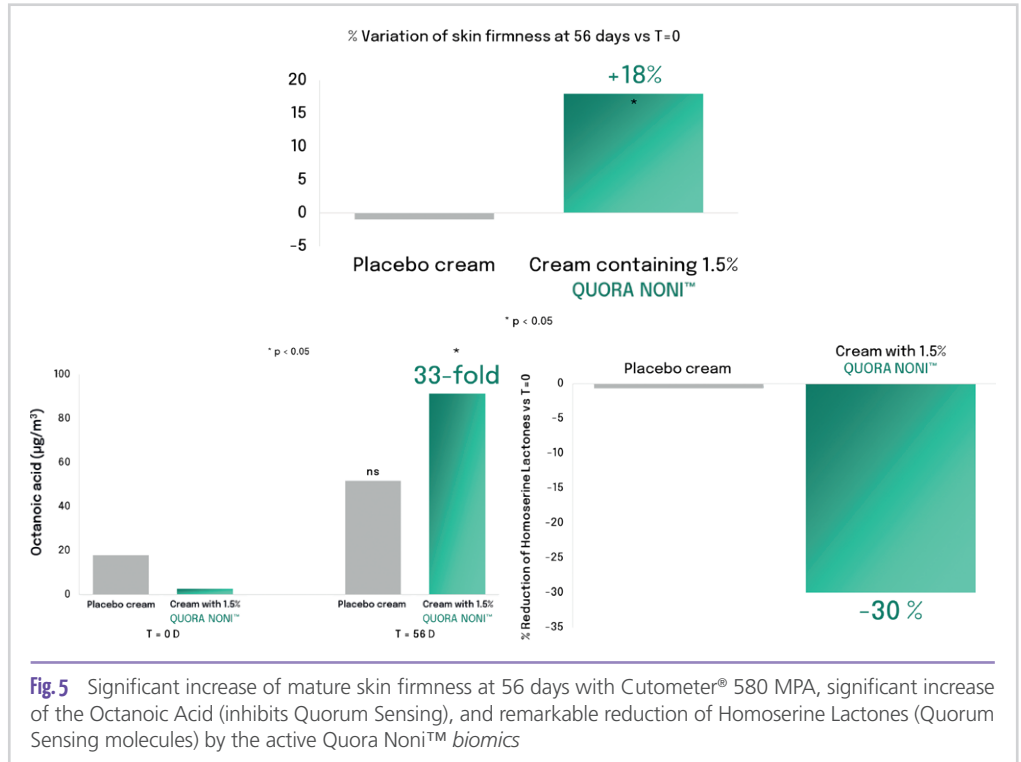


reduced, significantly, the length and the area of the Crow's Feet, by 8% and 9% respectively, with maximum reductions of 21% on the length and of 23% on the area.

**In vivo 2:**

Performed with 26 volunteers between 50 and 56 years old, double blind and placebo-controlled (hemi-facial applications), at the dosage of 1.5% of Quora Noni™ *biomics*, with two daily applications for 28 and 56 days.

The skin firmness was analyzed through cutometry (Cutometer® 580 MPA, Courage + Khazaka), at the beginning and at the end of the study (Figure 5). Furthermore, the general aspect of mature skin appearance was observed at the beginning of the assay and at 28 days using VISIA® (number of Crow's Feet wrinkles, red spots and pores, Figure 6).



**Fig. 5** Significant increase of mature skin firmness at 56 days with Cutometer® 580 MPA, significant increase of the Octanoic Acid (inhibits Quorum Sensing), and remarkable reduction of Homoserine Lactones (Quorum Sensing molecules) by the active Quora Noni™ *biomics*

Finally, in two different subgroups of 5 volunteers each, for the first time *in vivo*, the octanoic acid (inhibits Quorum Sensing) and the Homoserine Lactones (bacterial Quorum Sensing molecules) were quantified from skin biopsies at the beginning and at the end of the assay. The active significantly

increased the octanoic acid, by 33-fold, and remarkably reduced the Homoserine Lactones, by 30%, thus inhibiting the Quorum Sensing *in vivo*. (Figure 5).

The active significantly increased the firmness on mature skin at 56 days, while at 28 days a skin perfecting effect was already observed in these profiles, reducing the number of wrinkles, the pores and the red spots on the face of the volunteers.



**Fig. 6** Visible reduction of Crow's Feet wrinkles, pores and red spots at 28 days with VISIA® by the active Quora Noni™ *biomics*

## Conclusion

The active ingredient Quora Noni™ *biomixs* inhibits the Quorum Sensing, both *in vitro* and *in vivo*, reducing the SBMs and rejuvenating the skin microbiota.

This brings a new approach to skin care, in which we can recover a younger, healthier look without imperfections by rebalancing the cutaneous microbiota.

With this active ingredient from Noni stem cells and 100% natural and COSMOS-approved, we obtain a well-ageing effect through the rejuvenation of the skin microbiota: reduction of wrinkles, firmness increase, reinforcement of skin defenses, and mature skin improvement regarding the red spots and the pores.

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# An Instant Lift for Skin through the Power of Algae

R. Campiche, F. Pascucci

## abstract

Is there a way to deliver the instant skin lifting effect many consumers are looking for while also meeting their expectations for clean, eco-friendly ingredients? Our sustainably produced skin bioactive *Nannochloropsis oculata* Extract, Pullulan (commercial name PEPHA®-TIGHT CB) was originally proven to reduce the appearance of lines and wrinkles with long term use. In this article, we describe our new *in vivo* study which showed how it can also exert perceptible skin smoothing and lifting benefits in the short term. Specifically, skin texture appears smoother within minutes of a first application, while improved firmness and skin elasticity result in a lifting effect which is still noticeable 12 hours after a second application. *Nannochloropsis oculata* Extract, Pullulan comes within the increasingly popular category of ingredients derived from micro-algae and is produced through a biotech process which puts less strain on the environment and natural resources. This is likely to enhance its appeal to consumers requiring clean beauty solutions that are good for their skin but also respectful of the planet.

## Instant skin care benefits, sustainable ingredients, and the power of algae...

In today's fast-paced world, it is perhaps unsurprising that demand for beauty solutions that deliver instant results is on the rise. The skin ageing segment is no exception to this trend; so although many consumers are willing to invest in long-term use of a product to achieve a desired outcome (such as a reduction in the appearance of wrinkles), a growing number are looking for a more immediate, skin smoothing effect.

People are becoming equally uncompromising about their desire for "clean" products featuring naturally derived ingredients that can help promote healthy skin. One outcome of this expectation has been a burgeoning interest in the beauty benefits of algae extracts. Able to survive under the stress of different environments, algae are known for their resilience; and because they are rich in nutrients, such as vitamins, minerals, and antioxidants, they have long been considered a superfood – both for general health and the skin. With awareness of their skin care potential on the increase, face and neck care innovations featuring algae have grown at double the rate for the market average in the past five years [1].

Some years ago, DSM launched a sustainably produced skin bioactive derived from the microalgae *Nannochloropsis oculata* to meet the needs of the rising number of consumers looking for well-ageing skin care solutions that can target the appearance of fine lines and wrinkles. This ingredient (INCI name *Nannochloropsis oculata* Extract, Pullulan, Commercial name PEPHA®-TIGHT CB) is already proven to help improve skin elasticity and reduce wrinkles in the long term, through regular application. To respond to the more recent trend for instant

results, we therefore decided to investigate its ability to deliver short-term smoothing, firming, and lifting benefits as well.

## Could an extract of *Nannochloropsis oculata* change skin appearance immediately?

*Nannochloropsis oculata* Extract, Pullulan is a combination of purified microalgae active components and a high-performance polysaccharide, Pullulan, derived from fermented tapioca starch. The microalgae in question, *Nannochloropsis oculata*, lives naturally in both marine and freshwater lakes. However, for the purposes of our ingredient, it is cultivated in special photo bioreactors which provide optimal growing conditions for pure and high-quality production while also respecting environmental biodiversity. This biotech approach places less strain on the planet's limited natural resources – a factor which more consumers are beginning to consider when adopting more sustainable habits [2].

In their *in vitro* studies, our scientists showed how our bioactive can help reduce the appearance of wrinkles and improve skin elasticity by stimulating the formation of Collagen 1. With age, collagen density in the dermis decreases and its network becomes increasingly fragmented, presenting shorter and less organized fibres. Moreover, an increase in Matrix Metalloproteinase (MMP) expression accelerates collagen degradation while in parallel, synthesis of new extracellular matrix components by dermal fibroblasts decelerates, meaning that the degraded matrix is not adequately replaced [3,4]. Our active is designed to counter these processes and as the graph in **Figure 1** shows, *in vitro*, it is proven to increase cell proliferation in human dermal fibroblasts by +350% and to



stimulate Collagen 1 synthesis in human dermal fibroblasts by +70%.

This action is borne out by *in vivo* studies which show that in the long term, *Nannochloropsis oculata* Extract, *Pullulan* can visibly reduce wrinkle length by over -20% when applied on the face twice daily for 2 months at a concentration of 3% in a cream.

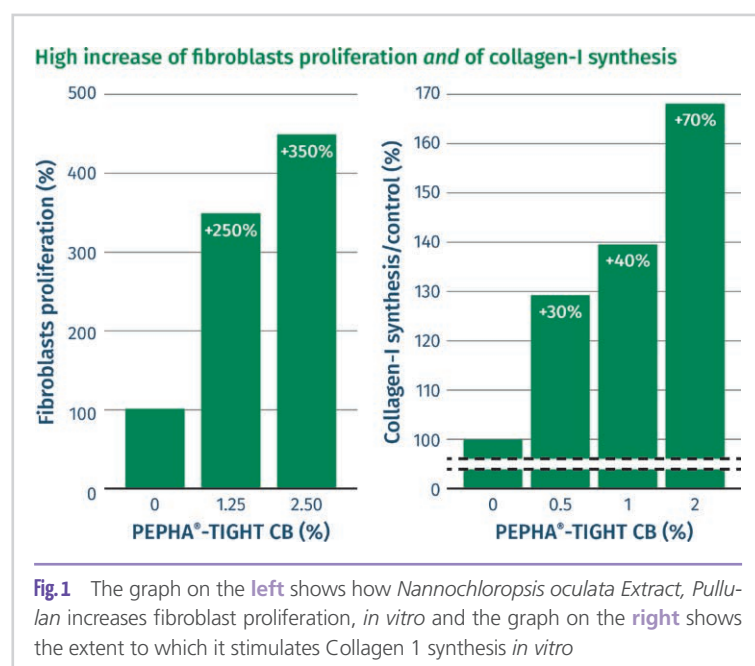
As well as stimulating Collagen 1 synthesis in the long-term, our active also forms a thin film on the skin immediately after application, thanks to the presence of *Pullulan*. Our new *in vivo* study therefore focused on its potential to deliver an immediate tightening and lifting effect

– something which many consumers desire for specific occasions when they want to look their best.

### New *in vivo* study focusing on short term effects

#### Study design

Our randomised, double-blind, mono-centre study involved two groups of 27 Caucasian female volunteers aged 40-65 years (average 55 years). One group applied a formulation with 3% *Nannochloropsis oculata* Extract, *Pullulan* twice daily in the morning and evening, and one group applied a placebo formulation twice daily in the morning and evening.



Measurements, to assess skin texture, skin firmness, and gains in skin elasticity were taken on various facial areas at the baseline (T0), within 15 minutes of the first product application (T Imm) and one day later, 12 hours after the second product application (T1). The different equipment used to measure the three criteria at different time points are described alongside our findings below.

#### Instant improvements to skin texture and its appearance

To show the immediate beneficial effect that *Nannochloropsis oculata* Extract, *Pullulan*, has on skin texture, we took images of each subject's skin surface, at T0 and T Imm (15 minutes after first product application) using a state-of-the-art, high-resolution

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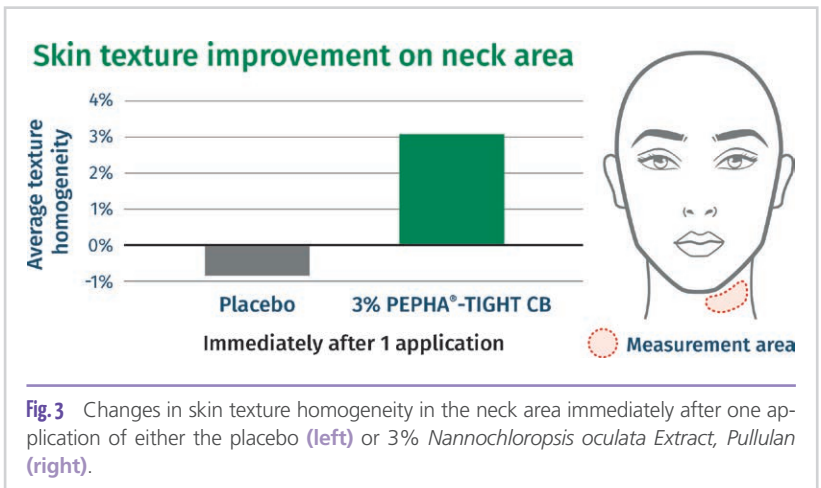




**Fig. 2** (Left) microscope images taken with a SkinCam show how irregularities in the skin's texture are less noticeable immediately after one application of *Nannochloropsis oculata* Extract, Pullulan compared to the baseline. (Right) photographs of the skin's surface, taken with a SkinCam, show how it appears smoother and has a more homogenous texture, immediately after a first application of *Nannochloropsis oculata* Extract, Pullulan.

Skin Cam. Images taken with the SkinCam device (Newtone Technologies, Lyon, FR) for four different volunteers are presented in **Figure 2**.

The images on the left depict changes as seen using parallel polarized lighting followed by a 3D-reconstruction of the skin surface, and those on the right are photographs of the skin's surface taken with cross polarized lighting. Both sets of images show that there was a visible improvement in the skin's texture, with the surface appearing smoother and more homogenous, immediately after first applying our bioactive.

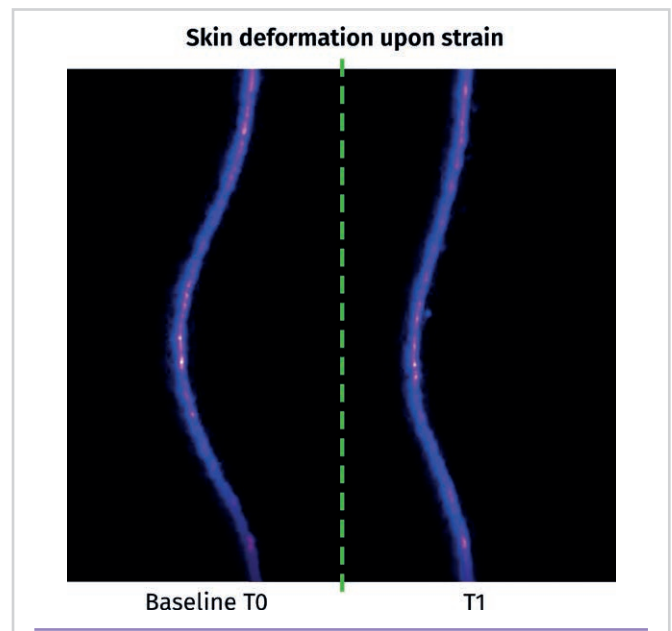


**Fig. 3** Changes in skin texture homogeneity in the neck area immediately after one application of either the placebo (left) or 3% *Nannochloropsis oculata* Extract, Pullulan (right).

We also represented SkinCam measurements for different facial areas in graph form. **Figure 3** shows changes in skin texture on the neck area after first applying either a placebo formulation or a formulation containing our ingredient and as can be seen, the use of 3% *Nannochloropsis oculata* Extract, Pullulan improved skin texture four times as much as the placebo.

### A long-lasting lifting effect

To assess longer lasting changes in skin firmness, our scientists took measurements using a SkinFlex device and software at T0 and T1 (12 hours after the second product application). The SkinFlex device produces an air stream (representing strain) which is blown perpendicularly to the cheek, pushing the skin inwards to form a "hole". The firmer skin is, the more able it is to resist the air stream. The impact of this process is illustrated by the images in **Figure 4** which also show the visible increase in the skin's resistance in one of our volunteers 12 hours after applying *Nannochloropsis oculata* Extract, Pullulan for a second time.



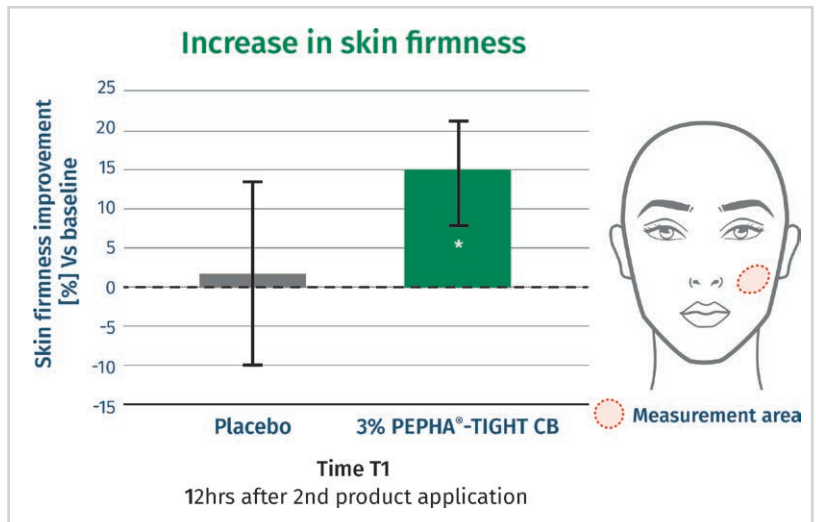
**Fig. 4** Software representation of SkinFlex measurements of skin's resistance to the airstream at the baseline, T0 (left) and 12 hours after a second application of *Nannochloropsis oculata* Extract, Pullulan, T1 (right). At T1, this volunteer's skin is visibly more able to resist the airstream

We then converted these SkinFlex measurements to percentages and presented them in the graph form shown in **Figure 5**. As can be seen, 3% *Nannochloropsis oculata* Extract, *Pullulan* proved to be 13 times more effective at increasing skin firmness, compared to the placebo, with these effects still being visible 12 hours after the second product application.

### Increased skin elasticity in critical facial areas

To assess gains in skin elasticity, 12 hours after the second product application, we took cutometer measurements in critical facial areas including the jawline, nasolabial fold, and eyelid. A cutometer works by applying a vacuum to the skin, pulling on it and then letting it go. As elastic skin bounces back to its original position fully and quickly, we calculate elasticity by measuring time, velocity and return to original position.

The graph in **Figure 6** shows the evolution of skin elasticity in the eyelid area at T1 for the placebo and 3% *Nannochloropsis oculata* Extract, *Pullulan*. In this facial area, the elasticity gain



**Fig. 5** Changes in skin firmness (compared to the baseline) in the cheek area, 12 hours after a second application of either the placebo (left) or 3% *Nannochloropsis oculata* Extract, *Pullulan* (right). \* p < 0.05 vs placebo

was six times higher with the bioactive than it was with the placebo.

We followed up our *in vivo* studies, by asking the volunteers about their own perceptions of using *Nannochloropsis oculata* Extract, *Pullulan*, via a questionnaire. When asked whether

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they agreed with the statement “my overall facial skin looks and feels firmer with *Nannochloropsis oculata Extract, Pullulan*”, 59% responded positively for immediately after application and 70% responded positively, for 12 hours after the second application.

### In summary

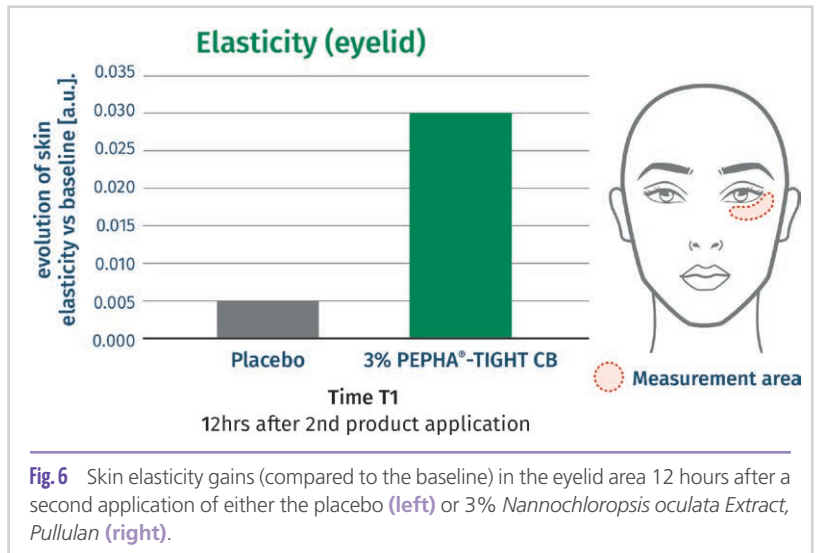
With a growing ageing population around the world, consumer demand for well-ageing skin care solutions is only going to increase. And beyond targeting the appearance of fine lines and wrinkles, people also want products that can help smooth, firm and lift their facial and neck skin. While many consumers are looking for longer term benefits in the solutions they use, others are looking for more instant results – a consequence of living in a fast-paced world, but also of a wish to look their best on specific occasions.

Our proven skin bioactive *Nannochloropsis oculata Extract, Pullulan* has been serving the needs of consumers wanting a long-term solution to reduce the appearance of lines and wrinkles for some time. Now, thanks to a new *in vivo* study, we can show that by forming a thin film on the skin, this ingredient can also exert a perceptible skin smoothing and lifting effect. Moreover, this effect can be felt instantly (within minutes of a first application) and is still perceptible 12 hours after a second application.

In addition to its proven skin care benefits, *Nannochloropsis oculata Extract, Pullulan* has the appeal of being derived from micro-algae, which are growing in popularity, and it is produced through a sustainable, biotech process. This would make it an invaluable ingredient for formulations offering consumers clean beauty solutions that are good for their skin but also respectful of the environment.

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**Fig. 6** Skin elasticity gains (compared to the baseline) in the eyelid area 12 hours after a second application of either the placebo (left) or 3% *Nannochloropsis oculata Extract, Pullulan* (right).

### Technical details:

**INCI Name:** *Nannochloropsis oculata Extract, Pullulan*  
**Applications:** Facial skin tightening products. Firming and lifting neck & décolleté formulations  
**Natural Index:** 100% natural origin content ISO 16128  
**Certification:** Cosmos approved. Verified by Ecocert  
 Vegan approved. Halal certified

### Formulation guidelines:

- Viscous, yellowish to amber solution
- Stable at pH ranges from 5 to 7
- Should be processed below 50°C
- Suggested concentration: 1–5%

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# Ceramosides™ HP, a Complex of Plant-based Ceramide for Well-aging

A. Momméja



## abstract

Aging has always been an important target for the beauty care industry. The way to talk about it and to address consumers has evolved though. In the 30's - 50's, aging was seen as an aggression and women needed to be saved from it. Between the 50's - 80's, there was a switch of mindset where images evolved to be more rewarding, with stronger & more independent women. In the 90's, anti-aging speech got militarized, as not showing the signs of aging was mandatory. In the 2000's, anti-aging evolved as campaigns showed more and more "mature" women seen as beautiful & seductive. In the 2010's, the anti-aging messages started to be supportive and encouraging. Aging was then also associated with environmental factors, such as pollution. Today, aging is seen as a positive token of experience. Traditional ways of marketing – specifically around age – are becoming less relevant. Women are growing more self-confident & are less anxious about the signs of aging. Well-aging has emerged. Well-aging is based on a healthy lifestyle and echoes healthy ingredients, well-known and reassuring for consumers, supporting and maintaining the healthiest skin possible. Ceramosides™ HP, a complex of phytoceramides, is one of these ingredients. Derived from wheat (nevertheless gluten free), a well-known plant, it is a plant-based ceramide whose benefits for skin are widely known, especially for mature skin. Interesting data were generated to support skin health and well-aging benefits.

## Composition

Ceramosides™ HP (**Table 1**) is a unique (patented) complex of phyto-ceramides similar to those found on the skin (ceramide 2, 3, 8). It also contains Digalactosyl Diglyceride (DGDG) that acts in synergy with ceramides as a performance activator. Ceramosides™ HP has a powerful antioxidant and elasticity supporting activity, bringing moisturisation and pore-minimising to the skin and eventually leading to self-esteem and positive emotions.

## Efficacy

### *In-vitro* tests

#### – Elasticity and firmness

Elasticity plays an essential role in the beauty of the skin. The skin has an "elastic capital", based on elastin fibers, that are degraded overtime and very seldom replenished. The loss of elasticity is irreversible and leads to the appearance of classical aging skin signs. Young skin explants were incubated for 1 hour at 37°C in a culture medium alone, to which we added either elastase or elastase + 0.005% Ceramosides™ HP. The skin incubated only with elastase demonstrated a degradation of -91.7% elastin fibers. The skin incubated with elastase and 0.005% Ceramosides™ HP demonstrated 47% protection of elastin fibers, meaning a reduction of -82.3% elastase activity. Thus, Ceramosides™ HP preserves skin elasticity by inhibiting elastase activity, an enzyme responsible for degrading elastin fibers in the dermal extracellular matrix.

Skin firmness also plays an essential role in the beauty of the skin and entails the organization in the extracellular matrix of collagen macromolecules and a proteinase equilibrium (ex: collagenase) / specific proteinase inhibitors (TIMP) which maintains the dermal matrix. With age, equilibrium between proteinase and their inhibitors is lost, resulting in excessive degradation of the dermal extracellular matrix. Tested on a fibroblast culture, Ceramosides™ HP boosts TIMP-1 synthesis by +33%. It is interesting to note that ceramides alone didn't act on TIMP-1 production. DGDG increased it, but only by 13%. Ceramosides™ HP thus demonstrated a true benefit of action thanks to its specific composition.

INCI	Glycosphingolipids & Glycolipids
Use level	0.2 -0.5%
Solubility	Oil & water dispersible
Description	Powder
Preservative	None
Claims	Improvement of skin complexion – Pore reducer – Positive emotions & self-esteem – Moisturization

**Table 1:** Ceramosides™ HP characteristics





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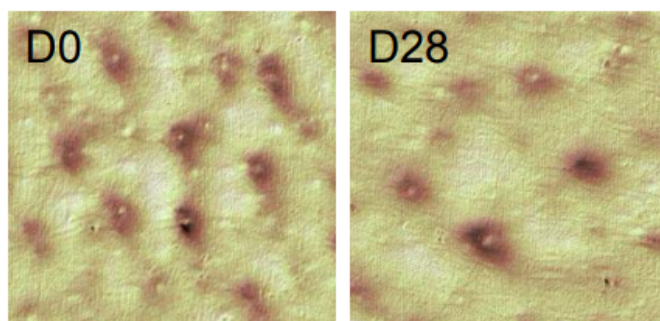
**In-vivo study 1**  
**– Pore minimizing effect and improved skin texture**

These elasticity protective effect and skin firmness support led to an *in-vivo* study measuring the pore-minimizing effect on mature skin. Indeed, with age, skin pores become enlarged and visible due to the cutaneous sagging: the diameter of pores is multiplied by 2 between the age of 25 and 50. Ceramosides™ HP was used at 0,2% in a cream and applied twice a day by 27 Caucasian volunteers with dull complexion & sagging skin during 28 days. Pictures of pores were taken by fringe projection.

On **Figure 1**, the result after 28 days shows that pores are diminished. Skin texture is smoother.

Changes in the quality of facial skin were evaluated by third parties as well. After 28 days of treatment with Ceramosides™ HP, 3 experienced judges measured significant improvement in skin quality vs T0. The complexion is clearer, fresher, more homogeneous, more matte.

The self-evaluation of volunteers revealed that skin is visibly more radiant for 70% of women, firmer for 74% of women and more toned for 78% of women.



**Fig.1** Images of the pore minimizing effect

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### In-vivo study 2

A second *in-vivo* study was realized on 53 volunteers with dull complexion & sagging skin. Ceramosides™ HP was used again at 0,2% in a cream and was tested versus placebo. The cream was applied twice a day. Volunteers were asked to self-evaluate their mood (mood scale by Mayer & Gaschke), to describe their physical appearance and to answer the question "What would be a stranger's impression of you?". The answers were processed by a neuroscience expert.

Like shown on **Figure 2**, in 28 days, Ceramosides™ HP at 0.2% had a positive effect on the volunteers' global mood. They express feelings of joy, increased calm and reduced feelings of sadness and nervousness. The extent of emotions is significantly different from that observed with placebo. Positive emotions are boosted. Furthermore, volunteers described themselves with more positive terms (better, less tired, change, love, radiant) and fewer negative terms than the placebo (before, don't like it, bizarre, nice, ugly, old), thus showing an improvement of self-esteem.

### Safety & application

Ceramosides™ HP is non irritant (eye irritation test) and showed a very good tolerance (skin irritation test). It is a powder better dispersible in water or oil at room temperature. It is the perfect active ingredient for well-aging cosmetic products: it cares for basic skin needs like antioxidant protection and moisturization (data not shown in the article but available

Emotions	Placebo T28 vs T0	vs T28	CERAMOSIDES™ HP T28 vs T0
Global mood	↘	<*	↗↗↗
Happy	↘	<*	↗
Calm	↘	<**	↗↗↗
Sad	↗	>*	↘↘
Nervous	↘	>*	↘↘↘↘

\*\*p<0.001 \*p<0.05

**Fig.2** Effect on the general mood of the volunteers

on demand), it supports skin elasticity and firmness against aging, it boosts positive emotions and self-esteem and improves skin texture. Finally, Ceramosides™ HP is a complex of phytoceramides that are widely well-known and reassuring for consumers to feel safe and trust it. All-in-one to support healthy skin and a more serene relationship with aging.

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# TENSAGEX Prime – Ether Sulfates with Optimized 1,4 Dioxane Content

B. Hoeltken, M. Zschiesche



Since January 2022 KLK Tensachem has been producing and supplying 1,4 dioxane optimized ether sulfates in different concentrations to interested customers all over the world. In conventional so-called rinse-off products, sodium lauryl ether sulfate (INCI: SODIUM LAURETH SULFATE, or in short form SLES) is often used as the primary surfactant. The SLES grades manufactured at the production site in Ougrée, Belgium using the latest technologies are characterized by a very low 1,4 dioxane content, which in turn allow customers to manufacture and market consumer products with the lowest 1,4 dioxane values.

KLK Tensachem is meeting the demand of customers who have asked for SLES with very low 1,4 dioxane levels following the introduction of a new regulation in New York State in 2019 [1,2].

Since negative effects on human health and the environment are considered likely, producers of ether sulfates have each been using the latest processes and technologies for many years to reduce the 1,4 dioxane content, which is unavoidably produced during the manufacture of ether sulfates, to a technical minimum.

With the latest innovation, KLK Tensachem was able to significantly improve the current market specification for 70% ether sulfates of <10 ppm with the production of TENSAGEX EOC670P-Prime with a guaranteed < 5 ppm (Figure 1). Here KLK Tensachem benefits from more than 30 years of experience in the production of different anionic surfactants to the advantage of the user.

After the introduction of new limit values in North America, the responsible authorities are also examining new rules for the European market. It is therefore advisable in any case to start look-

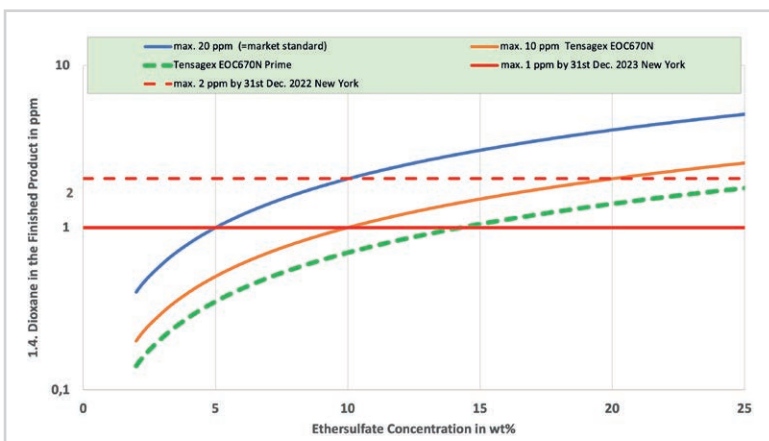


Fig.1 1,4 Dioxane-optimized Ethersulfates from the Prime series can be used for the manufacture of Home & Personal Care products with up to 15 wt% Ethersulfate

ing in detail today at the introduction of the next generation of ether sulfate products.

Based on this innovation, the TENSAGEX Prime portfolio has been developed (Table 1).

Product Name	Active Content	C-Chain Length	EO Grade	1.4 Dioxane (as such)
TENSAGEX EOC670P Prime	70%	C12-16	2 EO	< 5 ppm
TENSAGEX EOC670BP Prime	70%	C12-14	2 EO	< 5 ppm
TENSAGEX EOC626HPH-P Prime	26-28%	C12-16	2 EO	< 2 ppm
TENSAGEX EOC628HPH-P Prime	26-28%	C12-16	2 EO	< 2 ppm
TENSAGEX EOC628BVP Prime	26-28%	C12-16	2 EO	< 2 ppm

Tab.1 TENSAGEX Prime portfolio

As a responsible manufacturer, KLK Tensachem, as well as the entire KLK Oleo Group, is committed to sustainability. It is therefore a matter of course that all our surfactants are also available as RSPO-MB grades. Already since September 2021, the purchase of electricity and gas to produce ether sulfates is exclusively from sustainable CO2 neutral sources.

KLK Tensachem and Stockmeier Chemie have addressed the need for lower 1,4 dioxane values. In this partnership, a promptly deliverable solution with dioxane values < 5 ppm is presented to the market, in particular to cosmetics and home care producers.

*"The technological innovation TENSAGEX PRIME is aimed at all producers, currently with a focus on global manufacturers, who have already placed their products on the US market or would like to export them in the future safely"* commented **Bernhard Hoeltken**, Business Manager KLK Tensachem

The TENSAGEX Prime range is distributed in Germany, Austria, Switzerland and Poland by Stockmeier Group.

*"With the background of sustainability as well as product safety, we have been noticed an increased interest in the European market for years to reduce technically unavoidable by-products to new technologically possible minimums"*, added **Michael Zschiesche**, Director Life Science Europe at Stockmeier.

These surfactants have aroused your interest? We can support you in the formulation work for new products and will be happy to advise you on replacements in existing formulations.

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#### References:

- [1] The Laws of New York- Consolidated Laws of New York- CHAPTER 43-B Environmental Conservation- ARTICLE 37- Substances Hazardous or Acutely Hazardous to Public Health, Safety or the Environment- TITLE 1 Substances Hazardous to the Environment
- [2] The Laws of New York- Consolidated Laws of New York- CHAPTER 43-B Environmental Conservation- ARTICLE 35- Detergents and Other Household Cleansing Products

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# Green Chelating Agents for Industrial & Institutional Cleaning

A. Gripp, M. Heus, S. Muresan

## abstract

Formulators in the industrial and institution (I&I) cleaning market are challenged to develop more efficient systems such as low temperature wash for energy conservation, lower cost and /or with a more sustainable footprint. While keeping up with changing market dynamics they look to maintain or improve cleaning efficacy.

Selecting the right ingredients is important to meet user needs for performance and efficiency and this article will discuss the most effective green chelants available for I&I cleaning – glutamic acid diacetic acid (GLDA) and methylglycine diacetic acid (MGDA). Both are readily biodegradable or biodegradable under anaerobic conditions and perform at high pH where other green chelants may underperform. GLDA is the preferred option for liquid formulations while MGDA is preferred for solid applications.

## Introduction

The industrial and institutional (I&I) cleaning market was \$46.9 B in 2020 and is expected to grow 3.1% CAGR to \$54.6 B by 2025 [1]. The forecasted market growth is due to several factors including increasing attention toward environmental, social, and governance in which I&I plays an important role [1]. Further, more efficient systems such as low temperature wash are desirable due to energy conservation, lower costs and a more sustainable footprint. Formulators are challenged to keep up with these changing market dynamics all while maintaining or improving cleaning efficacy.

Selecting the right ingredients is important to meet consumer needs for performance and efficiency. Not surprisingly, surfactants are widely used ingredient in I&I cleaning and formulators want to be sure they are getting the most of their surfactant potential. Simply using more surfactant to do an adequate cleaning job is not optimal. For example, using higher amounts of surfactant can increase foaming which would require more water for rinsing and, therefore, is less desirable for a sustainable process. An effective way to improve surfactant activity is to use chelating agents (or known simply as 'chelants') which are often used to boost the cleaning effect.

The most effective green chelants available for I&I cleaning are glutamic acid diacetic acid (GLDA) and methylglycine diacetic acid (MGDA). Both are readily biodegradable or biodegradable under anaerobic conditions and perform at high pH where other green chelants may underperform. GLDA is the preferred option for liquid formulations while MGDA is preferred for solid applications.

## How chelants work

Chelants are organic chemical agents that interact with and change the chemical property of metal ions. The metal ion is then unable to attach itself to substrates such as fabric, tile, glass, metal etc. and is prevented from forming deposits on hard surfaces and/or stains on clothes. In essence, chelants work by binding metal ions that can cause hard surface deposits on just about any substrate and keep water 'soft,' a desirable attribute that usually helps with improved washing performance.

Chelants complex (bind) with multivalent ions, keeping the metal ion bound and preventing unwanted deposits.

By controlling metal ions, chelants also prevent precipitation of salts, extract metal ions to the aqueous phase (so they can be rinsed away), suppress metal catalyzed reactions, and reduce toxic effects of metal ions in formulations.

In practice the interaction between chelants and metal ions brings about the following effects:

- Prevention of precipitation
- Dissolution of precipitates and scale
- Fine-tuning of oxidation processes

Prevention of precipitation is important in water hardness control, micronutrients used in agriculture, and in other chemical processes. Dissolution of precipitates is advantageous when it comes to removing scale both in the cleaning and oil field markets. Control of metal ions is essential in either suppressing or accelerating oxidative processes. In the food preservation and pulp markets, traces of heavy



metal ions catalyze oxidation reactions resulting in undesired breakdown products.

The chelating agents we are focusing on in this article are aminocarboxylates. The combination of amino acid and carboxylic acid groups in each molecule give these chelating agents their superior qualities, enabling them to form highly stable chelates with metal ions.

The main characteristics of aminocarboxylates are:

- High affinity for metal ions over a wide pH and temperature ranges
- Good solubility in water
- Hydrolysis stability in both acid and alkaline media
- Good temperature stability
- Inert to most chemicals
- Low toxicity
- MGDA and GLDA are green chelates and are not persistent in the environment due to their good biodegradability and complete mineralization

It is worthy to note chelants may also be used to introduce metal ions into an application. In this case, the “chelant” changes roles to become a “chelate.” Although not usually desired for cleaning systems, there are applications that ben-

efit by having metal ions introduced. For example, chelates may deliver metal ions for food fortification, nutrients for plants, or act as a metal ion oxidizing or reducing agent.

## The role of chelants in industrial cleaning

### Textile cleaning

Chelants may help with textile cleaning in one of two ways. The first is control of heavy metal ions. Heavy metal ions need to be deactivated to safeguard unwanted decomposition of bleaching agent. The second is control of water hardness. The surface of the textile intended to be dyed can be affected by the hard water ions that prevent the penetration of dyes into the fiber.

### Cleaning and detergents

Chelants can be used as builders or for descaling. Builders reduce water hardness and ‘build’ the cleaning efficiency of the surfactant. Descaling in industrial cleaning (e.g., cleaning in place or “CIP”) is especially important for sugar plants, breweries, and dairy plants. Calcium-oxalate scale can develop in sugar plants and breweries while dairy plants can be impacted by calcium-phosphates scale. Chelants will work to descale both.

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Chelate	Pros	Cons	Special Features
<b>EDTA</b>	Most widely used, strong chelating agent; commonly used in detergents	Replaced in many applications due to slow biodegradability; Precipitates at low pH	Commonly used in detergents
<b>MGDA</b>	Medium to strong chelant strength, one of the greenest chelants available. "NTA-free"***; uses renewable feedstock		Offers efficiency (little is needed to be effective)
<b>GLDA</b>	Medium to strong chelant strength, one of the greenest chelants available. "NTA-free"*** and readily biodegradable; recommended for liquid formulations		May be offered as a 47% or a 55% active
<b>DTPA</b>	Strong chelate (not chelant)	Quickly precipitates at low pH; reprotox labeled (cat 2)	Mainly used as chelant in pulp and paper applications, it is a chelant. When DTPA is used as micronutrient in Agriculture (Fe-DTPA) it is a chelate.
<b>Glucosheptonate</b>	Readily biodegradable; sequesters iron at high pH; made from renewable feedstock	Can be pricy	Recommend GLDA or MGDA for greater cost efficiency
<b>NTA</b>	Inexpensive, good builder	Labeling required as NTA may cause cancer	GLDA or MGDA is recommended as a replacement
<b>HEDTA</b>	Strong chelating agent more soluble than EDTA at low pH, better in stabilizing iron at high pH	Highly soluble in more acidic conditions	Traditionally used in boiler cleaning and the oil field; also, recommended for gas scrubbing
<b>EDG</b>	Readily biodegradable	weak chelating agent, labeled carcinogenic (cat 2)	
<b>Citrate</b>	Good sustainable story	Not a strong chelate. Best performance at acidic and neutral pH. At higher pH, it loses efficacy. Mostly used for Calcium and Magnesium control.	MGDA is recommended as a sustainable substitute or salt formulations, for liquid formulations, we recommend GLDA
<b>STPP</b>	Use of phosphate restricted in some applications due to eutrophication* May be replaced with MGDA or GLDA	As a phosphonate, STPP is not highly desirable	

\* Restriction in the EU for home laundry detergents and home automatic dishwashing, \*\* Less than 0.1% NTA present for liquid GLDA and MGDA. Less than 0.2% for solid forms.

Tab. 1 Chelant comparisons

### Comparisons of sequestering builders in detergents

There are different types of builders that can be used in detergents. This section will focus on comparing sequestering builders by pros, cons and any special features. The comparisons are listed in [Table 1](#).

### GLDA and MGDA are the preferred green chelants

GLDA and MGDA are identified as green chelants because they meet the criteria for this classification. They are readily biodegradable and result in complete mineralization, come from (partly) renewable feedstock, meet Eco-label requirements (for Europe), and their NTA content is <0.1% for liquid forms. Citrates, also green chelants, function better at lower pH, and, therefore, may be less desirable for certain applications, especially laundry and hard surface cleaning.

### Stability constants indicate the strength of chelant/metal ion bonds

Stability constants (K) help identify the strength of a chelant bond. The higher the stability constant, the stronger the bond. Chelating agents can be selective for certain metal ions and can be pH dependent. Further, chelating capacity depends on the size of molecule but suffice it to say the smaller

the molecule, the less amount of chelant is needed and this can be an advantage for cost in use.

### GLDA and MGDA vs citrate and phosphonates

The trend in the I&I industry is to go green. The preferred options are GLDA and MGDA due to their superior performance and stability at higher pH vs citrate (high pH is often used in I&I cleaning).

Looking at stability constant values (K values), GLDA and MGDA outperform citrate at pH >6 and, in contrast to phosphonates, are readily biodegradable even biodegradable under anaerobic conditions.

#### Hard water conditions

In hard water conditions the Ca<sup>2+</sup> and Mg<sup>2+</sup> ions play a dominant role.

Citric acid can be used to remove hard water scale and works well at lower pH (acidic condition, pH ≤6). At higher pH, used in most cleaning processes, GLDA and MGDA will outperform citric acid.

The high pH in cleaning (alkaline conditions) is preferred because it will boost the hydrolysis of fat.

The comparison on the efficiency of citric acid as chelating agent versus GLDA / MGDA is dependent on the pH-range that is representative for the application (often pH >8). Figure 1 shows that GLDA/ MGDA are the more powerful chelants at higher pH.

Additionally, both GLDA and MGDA are capable to sequester transition metals such as Mn<sup>2+</sup> or Cu<sup>2+</sup>. The presence of these transition metals has a negative impact on stain removal processes. The following graph (Figure 2) shows the conditional stability constants of GLDA for various metal ions.

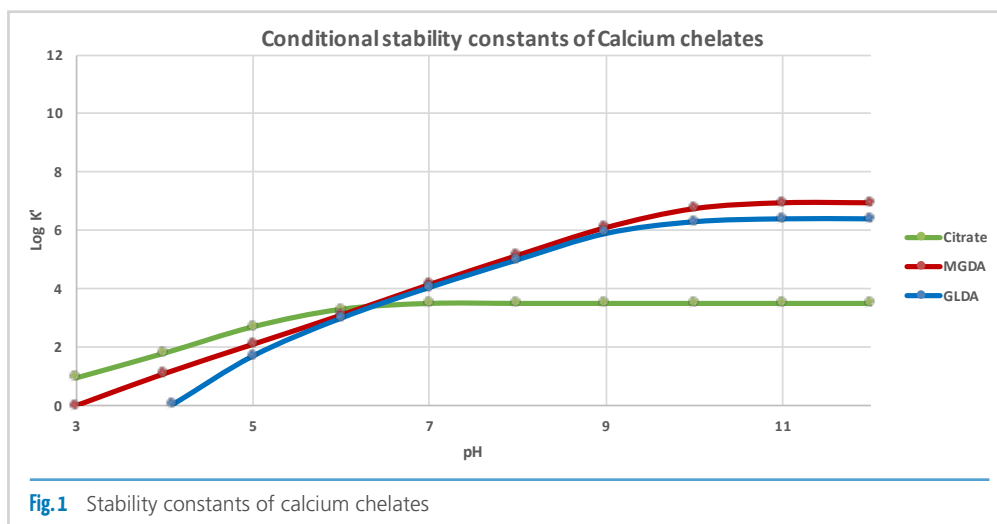


Fig.1 Stability constants of calcium chelates

### Effect of GLDA and MGDA

GLDA and MGDA show superior performance in dissolving hard water scale (CaCO<sub>3</sub>) compared to citrates, phosphates and IDS / phosphonate mixtures in an in-house laboratory test. The results in Figure 3 indicate the percentage of CaCO<sub>3</sub> dissolution at equivalent molar ratios of chelants tested.

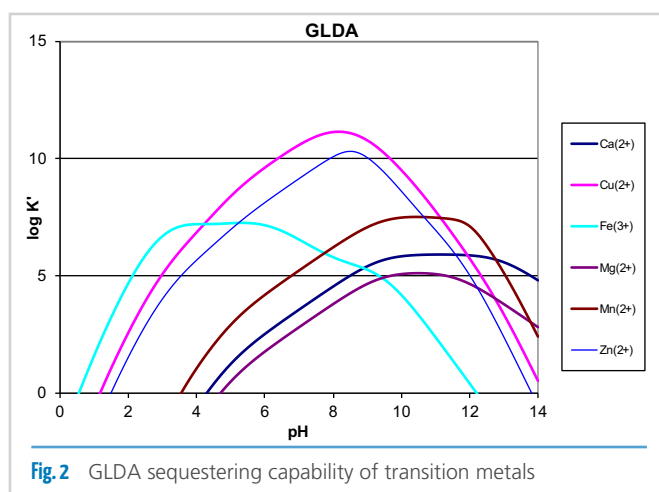


Fig.2 GLDA sequestering capability of transition metals

### GLDA and MGDA formulation guidelines

GLDA and MGDA are used in many cleaning products for both Household and I&I applications. The overall effect is boosting of cleaning performance by their strong ability to complex ion metals. The level of chelant in a product can range widely and it depends strongly on the application and the specific functionality needed. In addition, when used together with a biocide, the chelants can boost their biocidal efficacy.

### GLDA as a Performance Booster for Laundry Detergents

Hard water and transition metals both have a negative impact on stain removal. In hard water, a high amount of chelant is required to soften water due to large amounts of calcium. However, transition metals can be controlled using low concentration of chelating agents (<2.5 wt%).

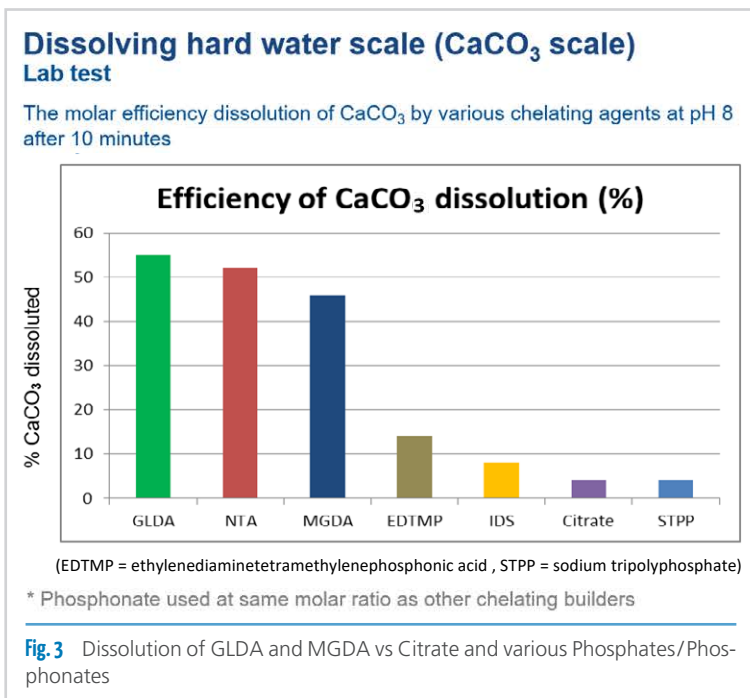


Fig.3 Dissolution of GLDA and MGDA vs Citrate and various Phosphates/Phosphonates

GLDA can control transition metals which is shown by enhanced stain removal for liquid detergents. It outperforms ci-

trate and has comparable performance but is bio-based and biodegradable vs phosphonate (DTPMP).



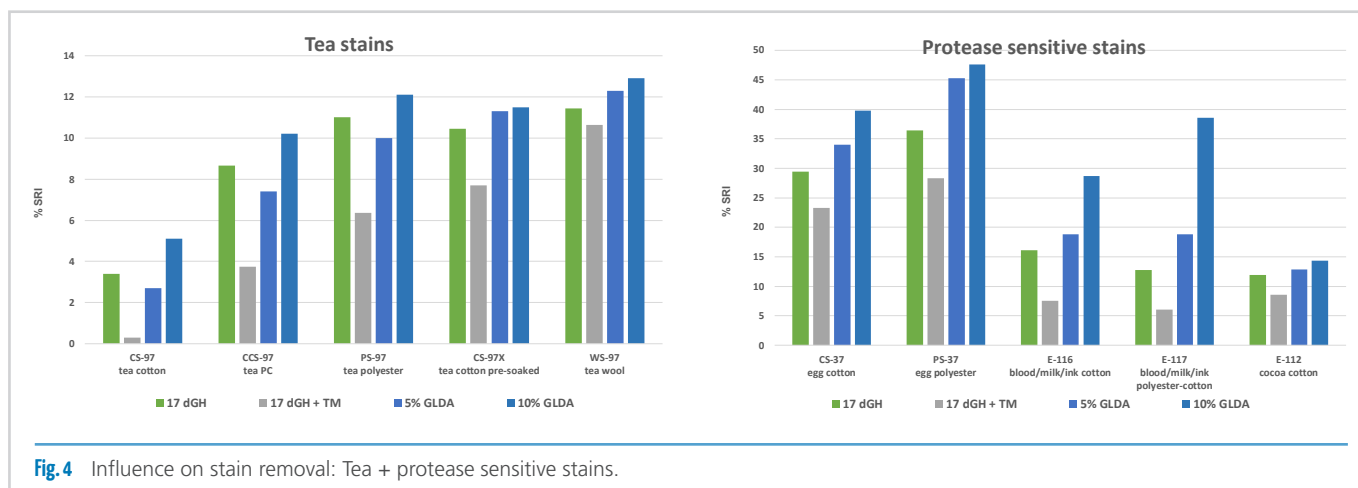


Fig. 4 Influence on stain removal: Tea + protease sensitive stains.

Figure 4 shows that addition of GLDA increases the stain removal index (SRI) and that GLDA improves removal of tea and protease sensitive stains even when used at levels lower than what would be required for hard water softening.

### Green chelates in strong degreasers for Hard Surface Cleaning (HSC)

Chelants are typically used together with surfactants in HSC I&I applications at high pH for strong degreasing capability.

To demonstrate the effect of green chelant MGDA vs citrate, an all-purpose cleaner was evaluated in a black-box cleaning test using diesel train soil applied on painted surfaces. Formulations were made with 10% surfactant 'as is' with 5% chelating agent active (MGDA vs citrate). The pH was adjusted to 10 and the formulations were tested at 1:30 dilution.

Based on the above study, MGDA is an optimal green chelant choice for removing diesel train soil vs citrate. GLDA shows equal performance compared to MGDA (Figure 5).

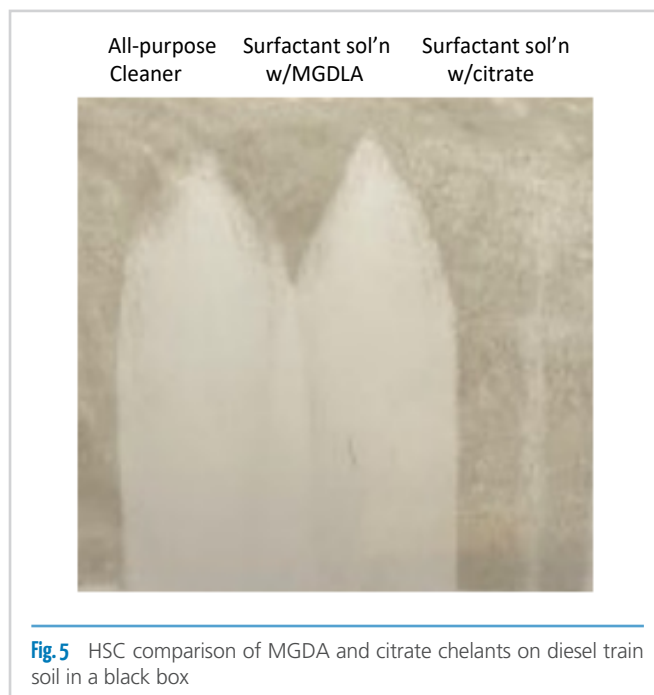


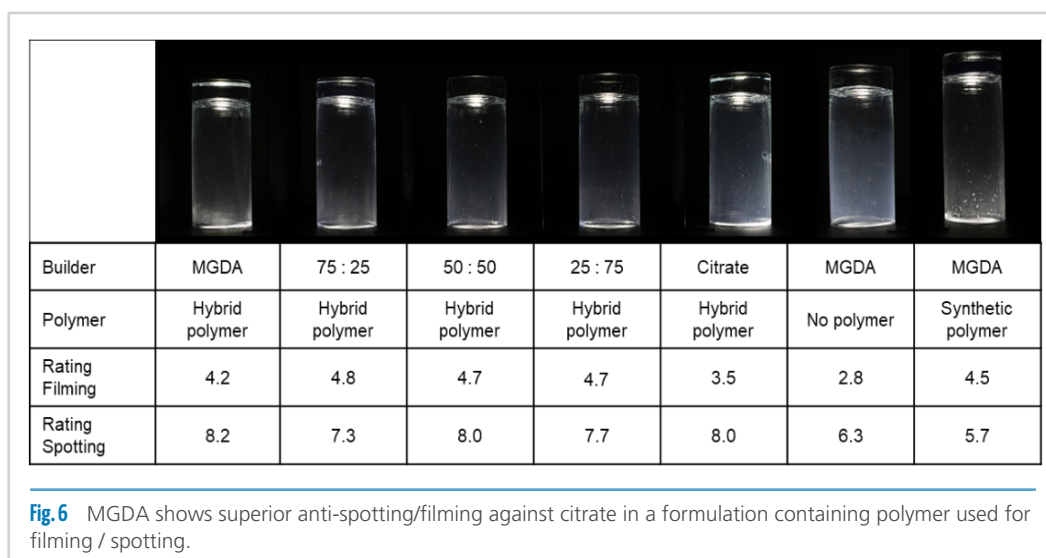
Fig. 5 HSC comparison of MGDA and citrate chelants on diesel train soil in a black box

Filming and spotting with MGDA are superior to Na-citrate. As expected, the polymer had a strong effect on filming (Figure 6).

### Green chelates in automatic dishwashing (ADW) formulations

Typical ADW formulations include a builder (chelant) and a co-builder (polymer) to address filming and spotting and ensure an overall good cleaning performance.

ADW formulations with 25% builder and 5% polymer were tested using EU test conditions.



## Conclusion

The challenge to I&I cleaning formulators is to keep up with the changing market dynamics for greener and sustainable solutions while improving cleaning efficacy. Ingredient selection is important. The most used ingredient in I&I cleaning is surfactant and formulators want to be sure the I&I cleaning market is trending toward greener formulation options. However, using more surfactant may not be ideal for sustainable results. Chelants can help boost cleaning efficacy and suppress scaling. The optimal green chelant options are MGDA and GLDA since they perform at higher pH v. options such as citrate which requires lower pH and is not ideal for many I&I cleaning applications. GLDA is recommended for liquid applications while MGDA is recommended for solid applications such as tablets in automatic dishwashers.

Both MGDA and GLDA are available in different concentrations. MGDA is available in either liquid or solid form. GLDA is available as a liquid in different strengths. Especially in the case of GLDA, a higher concentration form is recommended when lower water usage or lower amounts of solvents usage is a consideration for more sustainable applications.

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## Reference:

[1] Cleaners Industrial and Institutional – IHS – Dec. 2020

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## Oral Health Care with Jungbunzlauer ERYLITE®

Interview with Alina Böhringer, Technical Service Manager at Jungbunzlauer International AG



Alina Böhringer

***Erythritol is a well established sweetener in food applications. How does it find its way into oral care?***

Polyols are known for their positive impact on oral health. They have non-carbogenic properties since they cannot be metabolised by bacteria in the oral cavity. This is especially relevant for pathogenic bacteria responsible for oral biofilms such as *Streptococcus mutans*.

Several studies describe the effect and suggest mechanisms for more common polyols such as xylitol and sorbitol, but only recently included erythritol.

However, study designs differ much and results are hardly comparable. Now, we conducted our own *in vitro* experiments with realistic exposure times and polyol concentrations.

***According to your own results, did erythritol show an inhibitory effect on biofilm growth?***

Yes. We are happy to conclude that we see significant effects of erythritol. We see that a treatment with erythritol results in reduced numbers of colony forming units (CFU), which expresses a lower bacterial viability. Treated samples also show reduced bacterial membrane integrity in fluorescence microscopy. And last but not least, we measured reduced amounts of adenosine triphosphate (ATP) in a luminescence assay. This is an indicator of reduced metabolic activity of treated cells.

The results are comparable to those of xylitol in the same test setup.

***Please share some details with our readers. How were the experiments conducted?***

External *in vitro* testing was completed. In one approach, a "tooth brushing scenario" was mimicked, considering a usage level of erythritol in toothpaste or tooth tablets of 10 wt%, a 1:2 dilution by saliva and a typical tooth brushing time of three minutes. In a second approach, a "mouthwash scenario" was mimicked, assuming a concentration of 10%

of polyol in the mouthwash product, an undiluted application and a mouth rinsing time of one minute.

Biofilms were grown on substrates for several hours, then treated with erythritol or xylitol for a short time in the scenarios mentioned before and subsequently provided with sucrose to mimic sugar consumption after oral hygiene for some hours. Then, the evaluation was done with CFU counting, fluorescence microscopy and luminescence measures. Erythritol and xylitol were compared with each other and with a water control.

***What makes Jungbunzlauer ERYLITE® a valuable substance, especially in oral care?***

Our erythritol is considered natural because it occurs in nature and the production process is based on a non-genetically modified yeast fermentation of plant based raw materials. Erythritol is a versatile natural sweetener both in non-food and food applications.

In non-food application it is a multifunctional ingredient: in oral care it imparts a clean sweet taste and it has inhibitory effects on biofilm growth. Erythritol is formulated into toothpastes, mouth washes or tooth tabs. It is also used as low abrasive powder in tooth air-polishing. In personal care it is mainly used as moisturiser, in hair care it improves combability and foaming. In food applications, it works great as sugar substitution with zero calories and has a high digestive tolerance.

Erythritol is safe for use in animal care products, unlike xylitol. Like all Jungbunzlauer products, ERYLITE® profits from our sustainability initiatives to reduce greenhouse gas emissions and the like. Details are available in our annual Sustainability Report on our website [www.jungbunzlauer.com](http://www.jungbunzlauer.com).

***Would you like to add anything to conclude the interview?***

Watch out for our article titled «Positive impact of erythritol on oral biofilm» which will be published in March 2023 on our website. The article contains additional information and graphics on the test setup and results as well as interesting literature on the different mechanisms of action.

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**Water is an essential ingredient in many personal care products, and its quality can determine the quality of your products. If you are not testing your water systems regularly you could miss harmful organisms.**

With water being an essential ingredient in many home and personal care products, its quality can determine the quality of your products. If you are not testing your water systems regularly you could miss lurking harmful organisms like *Burkholderia cepacia* (*B. cepacia*). Since *B. cepacia* is a waterborne microorganism, this contaminant can be a reoccurring problem in your facility. Lack of data regarding the state of control of your water system poses a potential risk of missing objectionable microbial contamination getting into your products. Routine testing paired with accurate microbial identification of environmental isolates are essential because it catalogs the microorganisms that are resident or transient in your facility. Although water system monitoring is not required in cosmetics, water remains one of the top contamination sources found in manufacturing, so it is important that you are testing your system using validated methods on a regular basis.

Microorganism species belonging to the *Burkholderia cepacia* complex (*Bcc*) are opportunistic pathogens that have caused consumer illnesses and product recalls issued regulatory bodies. Water-based products that have been notably affected by *Bcc*-related recalls, include sanitizers, mouthwashes, skin creams, wipes, and baby products. *Bcc* is especially troublesome because of its abilities to resist preservatives and grow in unfavorable environments. One *Bcc* species, *Burkholderia multivorans*, can grow well in low-nutrient environments such as distilled water and can also form biofilms. Recently, the FDA found *B. multivorans* was detected in preserved nasal

spray prior to release. Two batches of the spray were discovered to be contaminated during microbial testing. Additional lots contained *Bcc* as well, though they previously had tested negative. The manufacturer determined that bacterial growth was initially inhibited by the preservative but then overcame it and flourished. Detailed testing identified the exact *Bcc* species responsible for the contamination was *B. multivorans*. Species level identification allowed the manufacturer to link the batch contaminations and perform a high-quality internal investigation to find the contamination source. Ultimately, the organism was traced to the purified water system and having established root cause, the manufacturer was able to correct the plumbing and implement additional corrective and preventative actions.

Any time part of your facility is offline due to a microbial contamination, it equates to lost profits. If the contamination arose from your water system, no amount of cleaning the manufacturing vessels will solve the problem because that is not the origin of the contaminant. An accurate species level identification can help link the microorganism source. The relative cost of accurate microbial IDs is small especially compared to the loss of inventory or a recall. With an environmental monitoring (EM) program in place, you can reduce the risk of this happening. EM programs will have you screening your water and other parts of your facility regularly and will serve as an early warning system that will save you time and money with faster and more effective contamination control.

Catching microbes before they become a problem and working to implement a microbial contamination control strategy means regularly monitoring your environment including your water sources with updated technology to catch microbial contamination faster and more accurately.



**Contact:**  
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Microbial Solutions, Charles River  
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Charlotte d'Erceville-Dumond

## Circular Beauty in Botanical Supply Chains

In an effort to save resources and avoid waste, Circular Beauty is a promising approach. In this interview, **Charlotte d'Erceville-Dumond**, Sustainable Innovation Manager at **BASF Beauty Care Solutions France S.A.S**, reveals what the term is all about, what challenges it poses, and which innovations BASF has developed in this area so far.

### **How do you define Circular Beauty?**

As the name suggests, Circular Beauty is based on the concept of circular economy. The principle is very

simple: the use or lifetime of a product is to be extended to the maximum by recycling, reprocessing and reusing waste as often as possible, thus turning it into the starting point for new products and processes.

However, the implementation is not quite as easy as the definition, especially in the beauty industry. Shampoo, shower gel and the likes are washed off after use, for example. The sewage sludge or the landfill are thus the final destination for Personal Care products. Nevertheless, we can ensure that their manufacture is as circular as possible.

There are many different benefits to this approach: low carbon footprint, energy and resource efficiency, net-positive products, local resources, and waste reduction. For us, in our botanical supply chains, Circular Beauty is about valorizing waste and upcycling materials. "Upcycling" in this context means that we utilize by-products of plants that usually go into waste to create new, valuable cosmetic ingredients.

However, the benefits of Circular Beauty go far beyond environmental protection by extending the life cycle of a product. It also offers crucial benefits for the people involved in the supply chains.

### **Why is BASF committed to this concept?**

For us, Circular Beauty is not just another trend that is in fashion today and forgotten tomorrow. It is quite the opposite – we see it as an important and long-term opportunity.

If we look at the numbers, strictly speaking, we are living beyond our means – or rather, beyond the means of Mother Earth. Our resources are finite. Today we need about 1.8 plan-

ets to provide the resources for our consumption and absorb our waste. And by 2030, we will already need two planets [1]. Needless to say, that we only have one.

That is why we attach great importance to manufacturing our products in the most resource-conserving way possible. Using botanical by-products is a very effective method that can help to save water, land space, agricultural inputs, and waste.

And more than that: In the end, circularity is not just about offsetting, but about creating positive impacts.

### **What do you consider to be the biggest challenges in Circular Beauty?**

One of the biggest challenges is also one of the most urgent requests from consumers: full traceability and transparency. Nowadays, it is no longer enough for people to know where a raw material comes from. They also want to know how it was planted, harvested and processed, and how the local population benefits from the trade.

To provide all this information, we rely on our great partners on site. It takes a fair amount of training of local farmers to achieve the expected quality and certification standards. Of course, this effort is only worthwhile for all parties if long-term contracts can be put in place.

A successful example is our rambutan program that was established in 2016. We utilize by-products of the rambutan fruit as feedstocks for active ingredients. Our partners in Vietnam organically cultivate the trees in the country's first rambutan plantation to be independently certified by Ecocert.

For our rambutan-based bioactive ingredients, we use the peels, seeds and leaves of the fruit. These are then further processed into skin and hair care ingredients. And that pres-

[1] <https://www.theworldcounts.com/challenges/planet-earth/state-of-the-planet/overuse-of-resources-on-earth> (last accessed 17 January, 2023)



ents yet another challenge, because the application areas have different business sizes and volume requirements. So, we need to carefully balance the demand of the different components within the circuit system.

And then, of course, the performance of the products plays the most important role. The use of waste and by-products is not an end in itself; it's about turning them into high-performance ingredients. That's where our R&D department does a great job.

***In the beginning, you said that the impact of Circular Beauty goes far beyond environmental protection. What did you mean by that?***

Using by- and waste products generates a new source of income for local farmers. Let's take the rambutan example again: Before we started producing cosmetic active ingredients from the seeds, peels and leaves, farmers depended only on the sale of the fruit, and the rambutan trees were at risk of being replaced due to market fluctuations. Using parts of the tree that would otherwise go to waste creates additional value for farmers, allowing them to invest in other agricultural value chains and incentivizing the preservation of rambutan plantations. Our program ensures income above average, gender equity, safer working conditions and health insurance. So, in addition to all the positive effects on the environment, Circular Beauty can also contribute to creating a positive impact on rural societies.

***You have already briefly introduced the Rambutan program. What other ingredients has BASF developed based on upcycling or by-product valorization?***

To date, we have about a dozen bioactives in our portfolio that were created through upcycling or by-product valorization of, for example, moringa oil cake, argan pulp, litchi peel and chestnut leaves. Their areas of application are as varied as their origins.

A new plant-based solution for hair care applications is the latest addition to our portfolio. This extract is the result of upcycling milk thistle seedcakes, a by-product of milk thistle oil production. The milk thistle we use is grown in France for oil production and is based on a traceable and sustainable supply chain.

Milk thistle oil is traditionally used in nutraceuticals and cosmetics. It is obtained by cold pressing from the seeds of *Silybum Marianum*. The leftover seed cake is rich in proteins, so we decided to use its potential for a new active ingredient. Our research team optimized the extraction process to generate low molecular weight peptides. The resulting amino acid composition of the product is close to that of keratin.



*BASF uses upcycled milk thistle seedcakes, a by-product of milk thistle oil production, to develop a new active ingredient for hair care applications. © GettyImages / PPAMPicture*

Keratin is a protein naturally present in the hair, which ensures its resistance and elasticity. Due to outer influences (environmental aggressions, lifestyle, styling, straightening, coloring), the proteins in our hair like keratin wear out and are depleted. The hair becomes dry, dull and brittle. To close this gap and rebuild the hair from the inside out, there are many products on the market that are enriched with keratin. The problem, however, is that the keratins that are usually contained in hair treatments are of animal origin.

Our new circular extract provides a sustainable alternative to animal keratin that makes hair stronger and helps keep its color longer. Comprehensive studies have shown that the active ingredient offers triple protection for the structural proteins of the hair against metal-catalyzed oxidation, glycation and carbonylation. The active ingredient also helps to prevent breakage especially of weakened hair through the stabilization and repair of hair keratin.

For me, it is always a marvel to see how we can develop such great products from materials that would have otherwise been disposed.

***Does Circular Beauty also play a role for BASF beyond plant-based active ingredients?***

It's true that we currently have the largest selection of Circular Beauty related products among our botanicals, but that does not mean we are not exploring these possibilities with other ingredients as well. Just recently, for example, we launched our new biopolymer Verdessence® RiceTouch, an upcycled plant-based sensory modifier made from 100% mechanically obtained natural starch.

I'm sure this is just the beginning. We will continue to research in this area to use our resources to the fullest in the most responsible way – and to help work toward a world of zero waste.

[www.basf.com](http://www.basf.com)



# DavosLife

## KLK OLEO

### Moisturising Hand Sanitiser with Super Vitamin E1

Phase	Trade Name (Product)	INCI	Supplier Name	% w/w
<b>A</b>	Ultrez 20.	Acrylates/C10-30 Alkyl Acrylate Crosspolymer	Lubrizol	0.25
	Water	Water	N/A	23.90
<b>B</b>	Alcohol	Ethanol	Fermpro	74.95
<b>C</b>	TEA	Triethanolamine	N/A	0.25
<b>D</b>	<b>DavosLife E3</b>	<b>Tocotrienols, Tocopherol</b>	<b>KLK OLEO</b>	<b>0.50</b>
	Beauty Blossom	Fragrance	N/A	0.05
<b>E</b>	Aloe Vera	<i>Aloe Barbadosensis</i> Leaf Extract	Terry Lab	0.05
	Aloe Vera	Maltodextrin	Terry Lab	0.05

#### PROCEDURE:

1. Phase **A**, Disperse Ultrez 20 into water.
2. Add in Phase **B** & follow by Phase **C** to form a gel.
3. Add in Phase **D** slowly into the mixture.
4. Finally add in Phase **E**, mix well.

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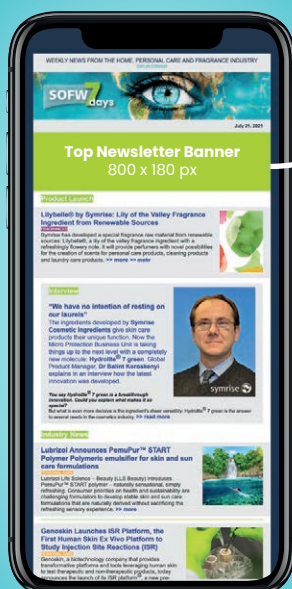
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HPCI POSTER  
AWARD



## HPCI CEE 2022 Poster Session Winner

### Warsaw | Poland

For the third time the Poster Session "Physicochemical aspects of creating stable cosmetic formulas" was organised by **The Warsaw College of Health and Engineering in Warsaw** as part of the **11<sup>th</sup> Home and Personal Care Exhibition and Conference (HPCI) Central and Eastern Europe\*** (21 -22 September 2022). The winning paper was awarded with this publication in **SOFW journal**.

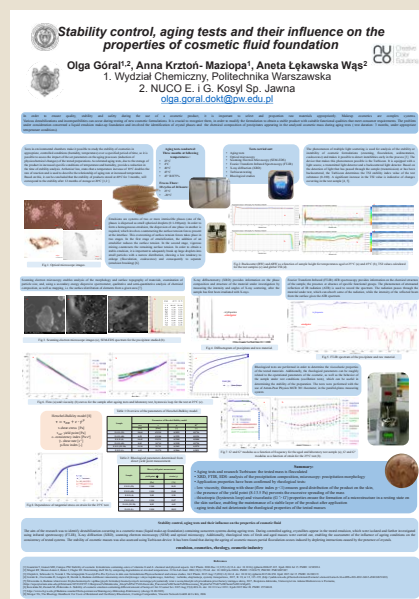
### Winning Poster

The winning poster was the research **Stability Control, Aging Tests and their Influence on the Properties of Cosmetic Fluid Foundation** prepared by **Olga Góral<sup>1,2</sup>, Anna Krztoń-Maziopa<sup>1</sup> and Aneta Łękawska Wąs<sup>2</sup>**

<sup>1</sup> Faculty of Chemistry, Warsaw University of Technology  
<sup>2</sup> NUCO E. i G. Kosyl Sp. Jawna

"The poster about stability control, aging tests and their impact on the properties of the cosmetic fluid, gives a good inside of the results. Charts along with descriptions and summaries allow the audience to follow the process of laboratory testing and methodology" – said Jury Member, **Agnieszka Nnolim** (Toxicologist, Nolichem Team). "The content of

the poster is in line with the title and covers the topic of stability otherwise known as the aging test with regard to cosmetic preparations. Overall, well presented and sourced poster."



Next HPCI CEE Poster Session will take place during **12<sup>th</sup> HPCI CEE** on **27-28.09 2023** in Warsaw, Poland.  
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Phase	Ingredients	INCI	Functionality	Quantity
<b>A</b>	<b>Crodamol™ ISIS.</b>	<b>Isostearyl Isostearate <sup>1</sup></b>	<b>Emollient</b>	<b>5.00</b>
	<b>SP Crodamol™ GTCC MBAL</b>	<b>Caprylic/Capric Triglyceride <sup>1</sup></b>	<b>Emollient</b>	<b>5.00</b>
	<b>SP Crodamol™ SSA MBAL</b>	<b>Decyl Isostearate (and) Isostearyl Iso-stearate <sup>1</sup></b>	<b>Emollient</b>	<b>5.00</b>
	<b>SP Cithrol™ PGTL MBAL</b>	<b>Tri (Polglyceryl-3/Lauryl) Hydrogenated Trilinoleate <sup>1</sup></b>	<b>W/O Emulsifier</b>	<b>2.00</b>
<b>B</b>	Water Deionised	Aqua	-	To 100
	Glycerin	Glycerin <sup>4</sup>	Humectant	4.20
	<b>Hydronesis®</b>	<b>Glycerin (and) Water (Aqua) (and) Salinicoccus Lysate Filtrate (and) Pentylene Glycol <sup>2</sup></b>	<b>Body Beautifier Active</b>	<b>3.00</b>
	<b>Crodarom® Amethyst GL</b>	<b>Water (and) Glycerin (and) Amethyst Extract <sup>3</sup></b>	<b>Skin revitalising active</b>	<b>1.00</b>
	Magnesium Sulfate Heptahydrate	Magnesium Sulfate Heptahydrate <sup>5</sup>	Emulsion stabiliser	0.80
	Microcare™ BNA	Benzyl Alcohol <sup>6</sup>	Preservative	0.80

Suppliers: 1: Croda 2: Sederma 3: Crodarom 4: Cargill 5: Sigma 6: Thor Specialities

**PROCEDURE:**

Combine Part **A** and Part **B** separately and stir each Part until homogenous. Slowly pour Phase **B** into Phase **A** whilst stirring at 600 rpm until fully combined. Homogenise for 1 minute per 100g at 10000rpm.

**TECHNICAL DATA:**

**Appearance:** White emulsion

**pH value:** N.A.

**Viscosity Brookfield:** 66,000cPs ± 10% (Brookfield DV2T Viscometer RV spindle 6, 10rpm, measured after 1 min at RT)

**Stability:** 3 months at 4°C, 25 °C, 40 °C, 45 °C, 1 month at 50 °C, 7 x -10 °C/+40 °C 24 hour freeze-thaw cycle



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**Description:**

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\* can be considered to be generally suitable for vegan; however, as there is no single agreed definition nor a standard, global certification, if you are interested in making a vegan claim, please request our Vegan Suitability statement for specifics on this product, and compare to the certification(s) you are seeking to meet.

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This formulation was developed in Europe. Contact your local sales representative with enquiries as ingredient availability can vary by region.

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IMCD <a href="http://www.imcdgroup.com">www.imcdgroup.com</a>	49	STOCKMEIER Chemie GmbH & Co. KG <a href="http://www.stockmeier.com">www.stockmeier.com</a>	37
in-cosmetics global <a href="http://www.in-cosmetics.com/global">www.in-cosmetics.com/global</a>	31	Symrise <a href="http://www.symrise.com">www.symrise.com</a>	Cover 4
IOI Oleo GmbH <a href="http://www.ioioleo.de">www.ioioleo.de</a>	29	TH.C.Tromm <a href="http://www.wax-tromm.de">www.wax-tromm.de</a>	Cover 3
JAKA <a href="http://www.jakabiotech.com">www.jakabiotech.com</a>	10, 17	Vytrus Biotech <a href="http://www.vytrus.com">www.vytrus.com</a>	21
KLK OLEO <a href="http://www.klkoleo.com/DavosLife">www.klkoleo.com/DavosLife</a>	9		
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