CefiraProtect CLR™ – Empowering skin's resilience

in vivo	ex vivo	in vitro
☑	☑	☑

CefiraProtect CLR™ acts as a daily companion for skin, protecting it against the influences of the exposome and making skin clearly more resilient.

It rejuvenates skin's own antioxidant potential and protects the most vital proteins of skin against free radical damage. In doing so, CefiraProtect CLR™ enhances the self-maintaining properties of skin, improving skin barrier, firmness and elasticity while counteracting in depth inflammations which drive skin deterioration with age.

Obtained from Lactobacillus kefiranofaciens, CefiraProtect CLR™ is a modern, vegan fermented skincare ingredient. Lactobacillus kefiranofaciens is an important constituent of kefir grains, and is known for its probiotic properties. It produces the high-value ingredient kefiran, a polysaccharide with significant health-promoting properties.

Dosage: 1.0 – 3.0 %
pH range: 3.0 – 9.0

INCI Name:
Betaine, Isomalt, Lactobacillus Ferment Lysate, Kefiran

CefiraProtect CLR™ is unpreserved.

Skin benefits

- Active vegan postbiotic obtained by Lactobacillus kefiranofaciens containing hero-ingredient kefiran
- Strongly activates cellular self-defense mechanisms against free radicals
- Skin-deep protein protection
- Long-lasting balancing effect against oxidative stress
- Regulates exposome-induced skin redness
- Improves skin barrier, elasticity and firmness

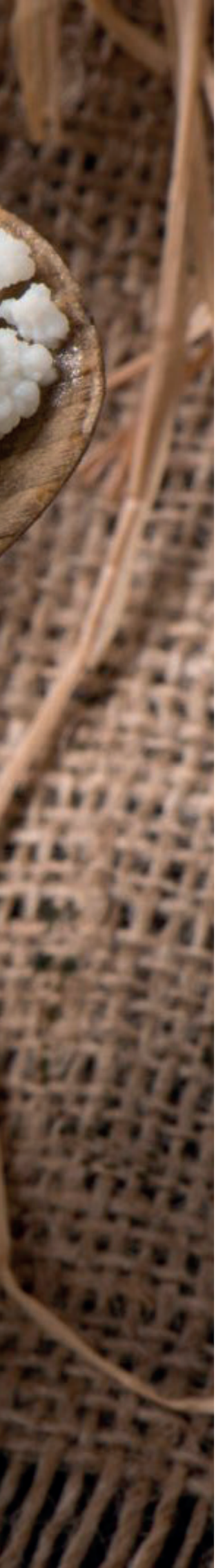
Applications

- Anti-wrinkle, firming, smoothing
- Skin regeneration
- Skin protection
- Soothing, calming, anti-stress
- Barrier maintenance and repair

Marketing opportunities*

- Vegan solution to activate cellular self-defense mechanisms
- Long-lasting balance against oxidative stress
- Guards against the cumulative impact of environmental factors on skin, keeping it looking fresh and vibrant
- Boosted firmness and elasticity for a more youthful and radiant appearance

* This list is for illustrative purposes only.
Make sure to comply with relevant legislation.



In recent years, there has been a growing awareness among consumers about the intricate relationship between the environment and their health, particularly their skin's. This collective realization has given rise to an increased interest in the concept of the exposome– the cumulative measure of environmental exposures and their effects on our bodies over a lifetime. Consumers are becoming more cognizant of how various elements of the exposome, such as pollution, UV radiation, diet, and stress, can adversely impact their skin's health and overall wellbeing. They have started to notice the visible signs of damage, such as premature aging, pigmentation, and loss of elasticity, and are increasingly concerned about the long-term implications for their overall health.

As a result, there is a burgeoning desire to understand and mitigate these influences. People are seeking out information and products that can help protect and rejuvenate their skin from the relentless assault of environmental stressors. This has led to a surge in the popularity of skincare products that boast protective and reparative properties, such as antioxidants, sunscreens, and barrier repair creams.

The impact of the exposome extends beyond just the skin, influencing systemic health and contributing to conditions such as respiratory and cardiovascular diseases. This broader understanding is driving individuals to adopt more holistic health and wellness practices. Lifestyle changes, such as improved diet, increased physical activity, and stress management, are becoming integral parts of their routines. The realization that a proactive approach can make a significant difference is empowering consumers to take control of their health and wellbeing. This growing consciousness is not only reshaping personal care routines but also sparking a broader movement towards a more holistic approach to health and wellness. The beauty and wellness industries are responding by innovating and evolving to meet the demands of informed consumers. As awareness continues to spread, the focus on the exposome and its impact on health is likely to become even more pronounced, heralding a new era of proactive, informed, and holistic self-care.

The skin and the exposome

We all know that skin is the first line of defense of our body against external stressors. This seemingly thin layer, where our inner world meets the often harsh environment, has the major task of keeping "bad things" out and "good things" in. Especially facial skin, arguably the part of skin which is exposed to exogenous threats the most, is constantly challenged by factors such as sunlight, pollution, etc., especially in the context of climate change, to which we are subject all over the world. Other factors which are essentially exogenous, such as psychological stress, lack of sleep and poor nutrition, also have a negative influence on skin. Altogether, these external factors are called the "exposome."

Facial skin is not just the part of skin which is most influenced by the exposome, its quality and visual features have a huge impact on how people look and are perceived by others. We all experience imperfections in our face. Blemishes, dullness, uneven pigmentation, sagging, formation of wrinkles, etc., more often than not are perceived to be a burden, and this is why the demand for effective and well-founded cosmetic ingredients is so high on the market. Finally, it is about preserving the skin as our stable shell, healthy, radiant and blemish free, no matter what life throws at us. In this context, a facial skincare product can be considered to be a "daily companion which keeps us in balance once we might start to waver." It is a product which helps people reach their skin goals: to maintain and, where possible, regain skin which makes them feel good and comfortable.

Oxidative balance at center stage

Facial skin is a special part of our skin, where maintaining or regaining its physical barrier properties alone does not suffice. Anything that penetrates the skin will have to be dealt with effectively on a molecular level. An important constituent of these added barrier properties of skin is its ability to deal with free radicals. Free radicals constitute a constant threat for our skin, especially the skin of our face, the place eyes meet first. Free radicals can be a product of external influences like UV or infrared radiation and air pollution. Even visible light is able to induce their production in skin. In addition, internal factors, such as deviations in normal metabolic processes, poor diet and psychological stress—all factors belonging to the exposome—lead to increased production of free radicals. For facial skin, the negative outcomes of the presence of too many free radicals are essentially innumerable. Indeed, skin is an organ which is tremendously dynamic and active. It is not just a "piece of leather" which protects our body. It is constantly trying to keep its natural and healthy balance. Oxidative stress, induced by the exposome, is compensated for by the skin's own cellular defense mechanism, with the aim to maintain its oxidative balance (see figure 1). Unfortunately, especially for facial skin which is so highly impacted by all members in the exposome family, it is a given that at a certain point the antioxidative cellular defense mechanisms of skin itself become exhausted. Looking at it as a constant fire that the skin is no longer able to extinguish on its own, it becomes clear why oxidative stress, and hence a permanent presence of free radicals, influences skin in many negative ways (see figure 2).

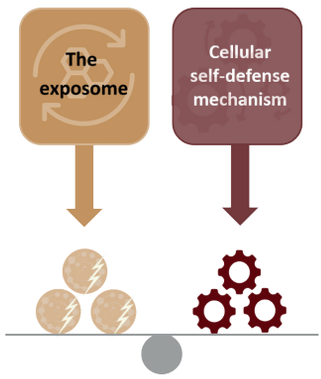


Fig. 1: Oxidative balance

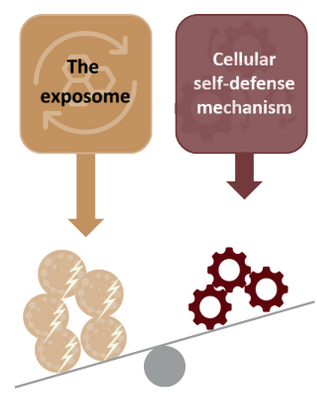


Fig. 2: Oxidative stress

The balance is tipping: free radicals are everywhere, especially in facial skin. They can lead to premature aging, where skin starts to lose its bouncy firmness and elasticity. It starts to sag, or wrinkles are formed. They play a role in inflammatory processes which make facial skin look unattractive and unhealthy, and they also play a role in uneven pigmentation. Skin can become dull, and free radicals can even have a negative impact on the pivotal role skin plays as a physical barrier.

Protein oxidation as a major focus point

Proteins are by far the most abundant type of molecules found in the skin. The keratins in the dead skin cells at the surface of our skin are proteins. The collagens and elastin lying in the deeper layer of our skin are proteins. The enzymes with which essential molecules are produced in skin are proteins. We could go on and on. All these proteins are prone to become oxidized in a situation where the skin has lost its ability to remain its oxidative balance. When they become oxidized, their molecular structure and spatial configuration change as well as their functionality (see figure 3).

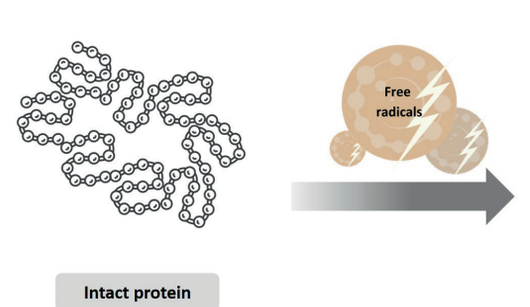


Fig. 3: Oxidized proteins as a consequence of oxidative damage

The oxidation of skin's proteins has dramatic outcomes for skin as it plays a central role in all features described above about the outcomes of oxidative stress and the overproduction of free radicals in skin. There is virtually no negative aspect of skin which does not include protein oxidation.

Skincare to the rescue?

A facial skincare product which can act as a "daily companion" for the consumer, supporting her effectively in maintaining or regaining good skin quality and looking attractive, must maintain or regain the skin's oxidative balance. Intuitively, using antioxidants in a facial skincare product is sufficient. Or is it? Most antioxidants are inherently unstable in cosmetic formulations. Depending on their dosage, they might even become pro-oxidative and essentially become part of the oxidative problem in skin. Moreover, the use of antioxidants can be considered to be merely a "band aid": they might still play a positive role, but do not provide a profound solution to the daily challenges to which facial skin is exposed.

CefiraProtect CLR™ – honoring traditions and bringing them into the modern age

A next-level solution is a facial skincare product which promotes oxidative balance in skin by boosting the antioxidative cellular defense mechanisms in skin in such a way that they do not become exhausted in the first place. If you want to take such a step forward, it can be helpful to look at which traditional ingredients and which old knowledge can help you to achieve this new way of thinking. Kefir is a traditional fermented milk beverage which has been around for several thousand years and is thought to have originated from the nomads of the Caucasus mountains. Its self-preserving and powerful antioxidant properties have made kefir a drink whose production methods have been passed down from generation to generation. When transferring that knowledge to skincare, is there a way to preserve the health-promoting properties of this source, to strengthen the self-regulatory powers from within and to keep the process vegan?

This is what CefiraProtect CLR™ provides. It is a product which is based on Lactobacillus kefiranofaciens, a species of lactic acid bacteria commonly found in fermented dairy products, particularly kefir grains, and is known for its probiotic properties. This bacterium plays a crucial role in the formation of kefir grains and is able to produce the high-value ingredient kefiran, a water-soluble polysaccharide with significant technological and health-promoting properties. To remove these beneficial bacteria from their usual dairy-based comfort zone and use them on a plant basis requires a very high level of technical expertise, something that CLR Berlin has stood for for decades. Inspired by tradition, this expertise results in a vegan postbiotic that employs advanced, sustainable, cell-based fermentation technology.

Efficacy studies: in vitro and ex vivo

To obtain sufficient evidence for the beneficial effects of CefiraProtect CLR™ and its relevance as a daily skincare companion, numerous in vitro, ex vivo and in vivo experiments were performed. The in vitro experiments were performed on 2D cultures of skin cells (keratinocytes). The objective of the first experiment was to find out whether CefiraProtect CLR™ is able to boost the cells' own antioxidant potential, their endogenous ability to reduce the elevated presence of free radicals which were induced by important stresses and highly relevant representatives of the exposome, UV-irradiation and a peroxide. Secondly, these in vitro experimental designs were extrapolated to ex vivo studies using skin explants, i.e., real and live tissue.

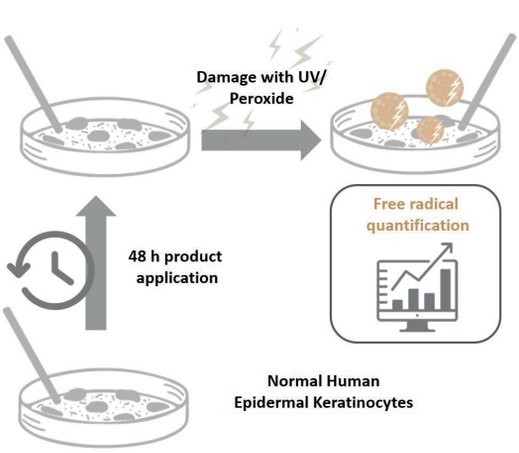


Fig.4: Test method for free radical quantification

In vitro: reduction of free radicals (ROS)

Normal human epidermal keratinocytes (NHEK) were incubated with test materials (control or CefiraProtect CLR™) for 48 hours. After washing the cells, they were exposed to either UV irradiation (2 J/cm² UVA / 0.2 J/cm² UVB), or 150 µM TBHP (tert-butyl hydroxyperoxide, for 30 minutes) to induce the production of free radicals. Subsequently the presence of free radicals was quantified with DCFDA / H2DCFDA—Cellular ROS Assay Kit (abcam; ab113851). Control was set at 100% (see figure 4).

Results: In both instances the different concentrations used showed that CefiraProtect CLR™ reduced free radicals / ROS formation by approximately 20% (see diagram 1).

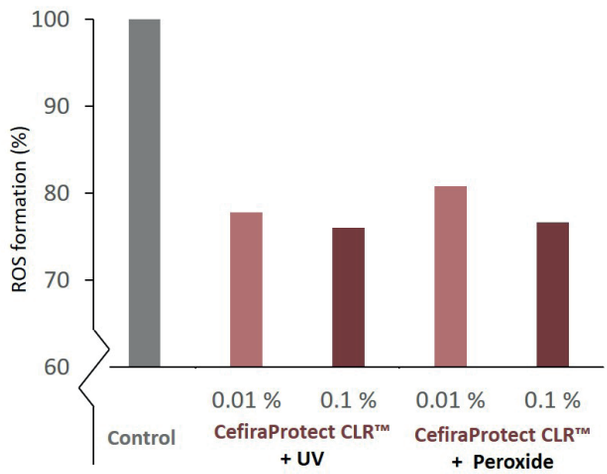


Diagram 1: Reduction of free radicals

Ex vivo: skin antioxidative potential

Skin antioxidative potential was measured by ESR (Electron Spin Resonance) spectroscopy. Skin was labelled with an ESR active test radical (TEMPO, 2,2,6,6-tetramethyl piperidine-N-oxyl, Sigma-Aldrich, Munich, Germany) that is reduced by the antioxidant systems inside the epidermis and dermis.

Method:

1. Test products were applied topically on skin explants.
2. After 5 hours: skin explants were irradiated with UVA/B (UVB (280–320 nm) at 23.5 W/m2 and UVA (320–400 nm) at 180 W/m2).
3. Explants were placed on a filter paper saturated with the test radical at 1 mM concentration in water for 5 minutes.
4. Skin biopsies of 4 mm ø were taken and placed in a special ESR tissue cell.
5. ESR spectra of the test radical were recorded and results generated.

Non-stressed and untreated control was set at 100% (see figure 5).

Results: For both skin explants it could be shown that CefiraProtect CLR™ helps to fully re-establish and even improve skin's antioxidant capacity. Interestingly, the placebo (vehicle, identical formulation but not containing CefiraProtect CLR™) showed to have a negative impact. This might relate to its negative effect on skin barrier (see diagram 2).

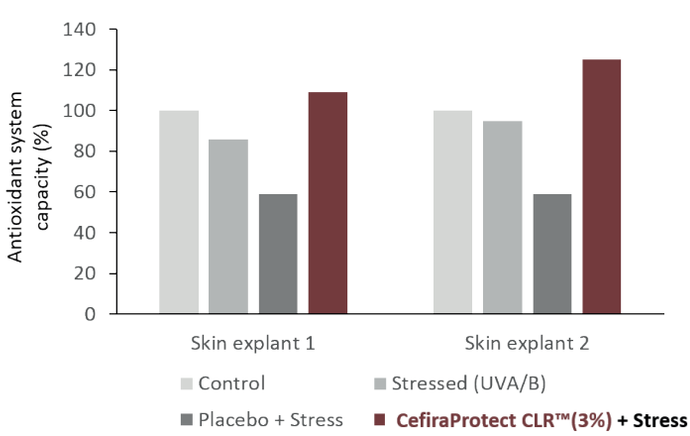


Diagram 2: Re-establishment of skin's antioxidant capacity

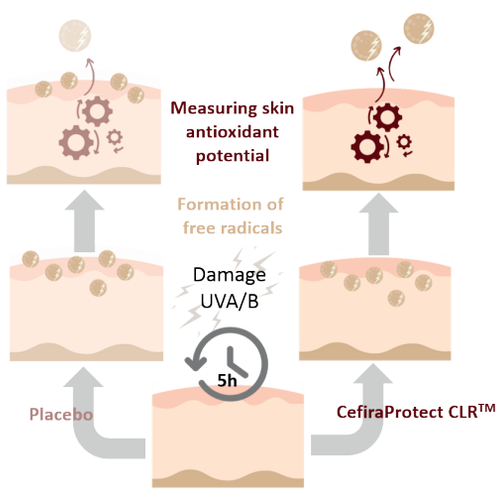


Fig. 5: Test method for measuring skin's antioxidant potential

In vitro: protection against protein oxidation

Normal human epidermal keratinocytes (NHEK) were incubated with test materials (control or CefiraProtect CLR™) for 24 hours. After washing the cells, they were treated with UVA irradiation (3.9 J/cm² UVA, 365 nm) to induce oxidative processes and protein oxidation. Subsequently quantification of protein oxidation took place as follows: cells were fixed to a multi-well plate using an Ethanol/Acetic acid solution. Carbonyls (products of protein oxidation) were labelled in situ with a functionalized fluorescent probe to specifically bind carbonyl groups (Baraibar, MA et al., J Proteomics, 2013 30;92:63–70). The excess of fluorophore was removed by successive washes with PBS. The specific fluorescence signal for each well was recorded using an epi-fluorescent microscope (EVOS M5000 Imaging System) and analyzed with ImageJ software (Schneider, 2012). Control was set at 100% (see figure 6).

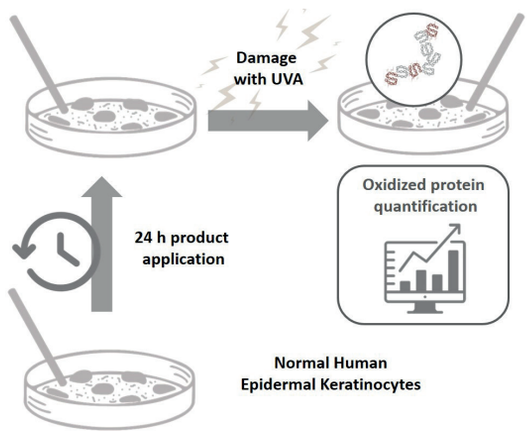


Fig. 6: Test method for oxidized protein quantification

Results: In this in vitro setting it could be shown that treatment with CefiraProtect CLR™ clearly reduces the presence of oxidized proteins (see diagram 3).

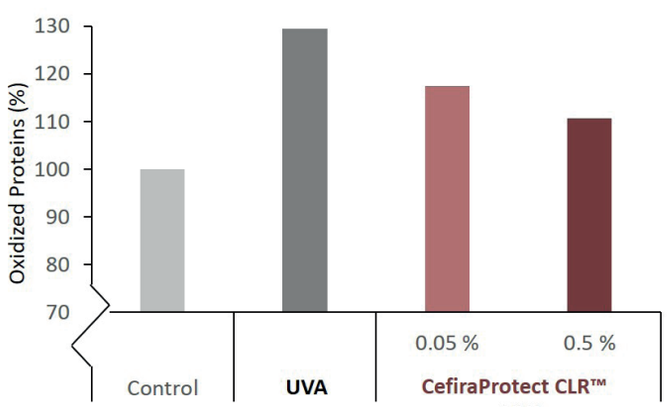
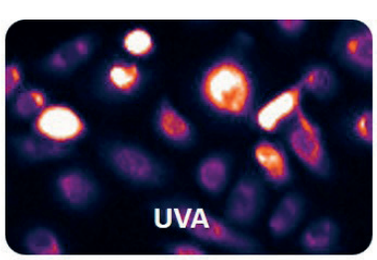


Diagram 3: Reduction of oxidized proteins

This is elegantly visualized in the microscopic pictures which show a noticeably lower level of fluorescence caused by oxidized proteins.



Ex vivo: skin-deep protection against protein oxidation

Explants were obtained from abdominal surgery. Donor: 41-year-old female Caucasian (phototype II/III).

Test products were topically applied on the skin explant's surface (2 mg/cm2) and explants were further incubated for 24 hours. Subsquently the skin explants were irradiated with UVA (365 nm): 6 J/cm² UVA, 40 minutes of irradiation. Then skin explants were sampled, transferred in OCT for cryopreservation, snap-frozen in liquid nitrogen, and conserved at –80°C until analyses. Finally quantification of protein carbonylation (as a product of protein oxidation) took place (see figure 7).

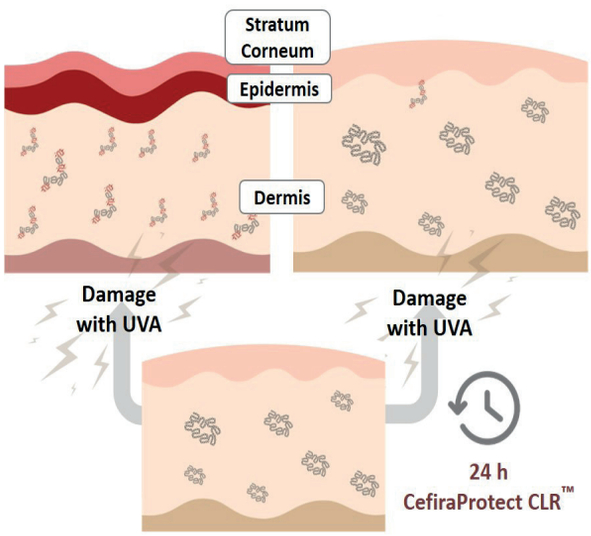


Fig. 7: Skin-deep protection against protein oxidation

Method of analysis: Explant sections with 5 µm thickness were obtained using a cryostat (Leica) and fixed with a solution containing 95% ethanol and 5% acetic acid. Oxidatively damaged (carbonylated) proteins were labeled using an OxiProteomics® fluorescent probe (Ex = 647 nm / Em = 650 nm) functionalized to specifically bind to carbonyl moieties and DAPI (4',6-diamidino-2-phenylindol) for nuclear labeling. Fluorescent images were collected with an epi-fluorescent microscope (EVOS M5000 Imaging System) and analyzed with ImageJ software (Schneider, 2012).

Non-stressed and untreated control was set at 100.

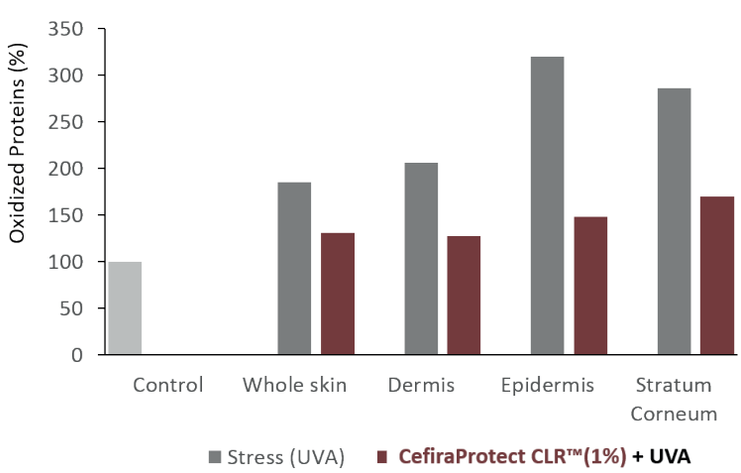


Diagram 4: Reduced presence of oxidized proteins in all skin layers

Results: The histological pictures of this ex vivo experiment reveal how dramatic the effect of UVA irradiation is and that the presence of oxidized proteins is very strongly increased in the stratum corneum, at the dermal–epidermal junction, and in the dermis (see figure 8). It could be shown that in all skin layers, treatment with CefiraProtect CLR™ strongly helps reduce the presence of oxidized proteins (see diagram 4). As is the case for the result obtained on oxidized proteins in the above-described in vitro study, the results from this study are coherent with the earlier studies which showed that CefiraProtect CLR™ improves the skin's own antioxidant potential.

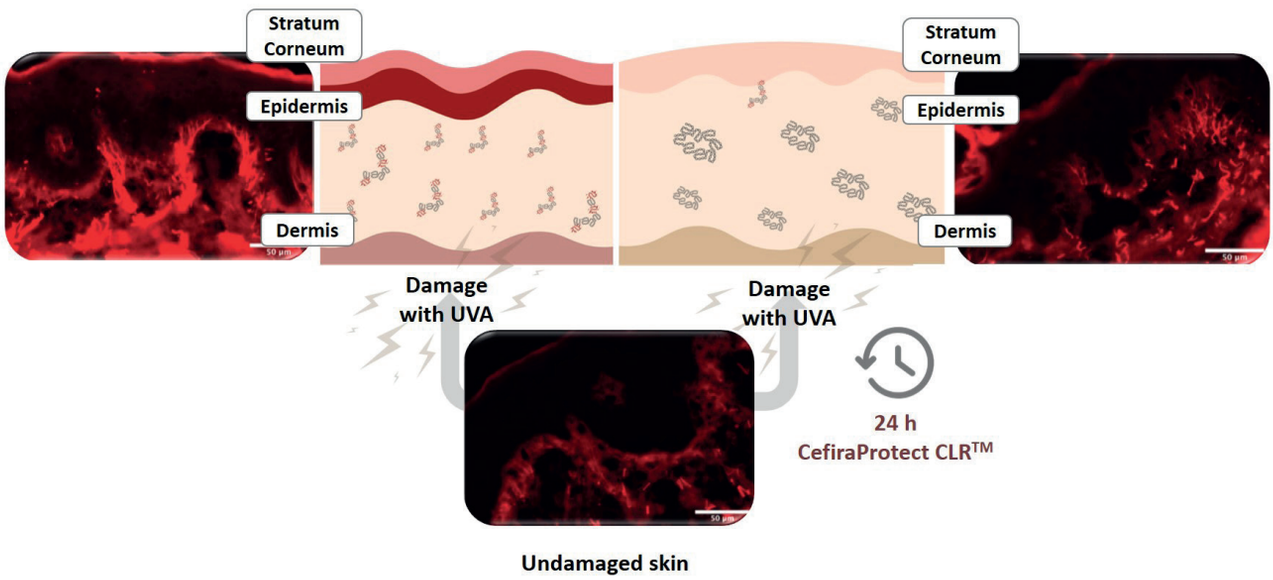


Fig. 8: In-situ visualization of skin-deep protein oxidation

Efficacy studies: in vivo studies

As was shown in the in vitro and ex vivo experiments, CefiraProtect CLR™ as an active ingredient for the daily care of facial skin provides oxidative balance. In vivo experiments (as described below) were subsequently performed to confirm the fact that CefiraProtect CLR™ was able to improve the recovery of skin after exposure to an exposome-relevant stress and its protective effects against this stress, in essence improving skin resilience against the exposome.

In a second batch of in vivo studies it was then investigated whether CefiraProtect CLR™ could improve important characteristics of skin which normally deteriorate consequential to its exposure to the exposome. These studies were performed in the context of the fact that resilient skin equals self-maintaining skin. Indeed skin, as our body's largest organ, is incredibly dynamic and constantly working on maintaining its qualities. Its barrier function, for instance, is not just important for skin's hydration levels and smoothness, but is also essential for our body as a whole.

Other typical and essential characteristics of skin, its mechanical properties, i.e. its firmness and elasticity, are similarly important for human life. Skin needs to be firm and elastic to be able to bend and regain its original shape while our body moves in all directions. However, skin firmness and elasticity play a very important role in many skin features related to skin aging. With age, skin loses its firmness and elasticity, which leads to the formation of wrinkles as well as skin sagging. Fully functional and resilient skin is able to maintain its mechanical properties even under the influence of the exposome.

Strongly related to the above is the realization that the exposome does not merely provoke the elevated presence of free radicals. The exposome induces a cascade of cell biological and biochemical changes in the skin, many of them related to free radicals, and many of them leading to an inflammatory state of skin which is chronic but necessarily visible or otherwise perceivable. We might call this "hidden inflammation." It is a type of inflammation which can have devastating effects on the skin in the long run, many of them related to typical features which are visible in aged skin, such as wrinkling, sagging, but also hyperpigmentation as seen in age spots.

The effect of CefiraProtect CLR™ on skin barrier function, "hidden inflammation," skin firmness and elasticity was assessed as well.

In vivo study 1: exposome-induced stress and skin resilience

A study was performed on the inner forearm of 13 women (average age 38.6 years). The study had three phases.

During the first phase, the so-called "irritation phase," no test products were applied, but skin was challenged twice a day with a 5% SDS (Sodium Dodecyl Sulfate) solution in water for a total of 7 days. During the subsequent 7 days, the "treatment phase," the skin was not stressed with SDS, but was treated twice daily with test products (CefiraProtect CLR™ (3%), Placebo, Untreated). During this second phase the SDS-induced irritation was reduced. The interest was to see whether there was a difference between the treatments in the speed with which effects occurred. In the third and last phase test products were applied twice daily. One hour after application of the test products skin was again challenged with 5% SDS. In this phase the purpose was to find out whether the treatment had an impact on the renewed exposure to SDS and whether the different treatments led to a difference in skin resilience. Skin redness was determined with a Minolta Chromameter CR 400 (Minolta, Japan) using the CIELab color space. Irritated skin shows a higher red component which can be quantified by the a* value. Each value is the average of three recordings. Base redness was set at 0% (see figure 9).

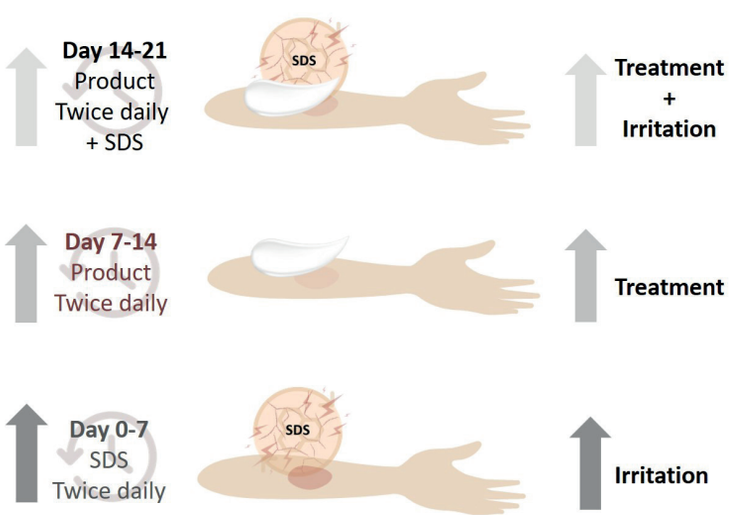


Fig. 9: Test method for measuring skin redness

Results: The initial "irritation phase" clearly showed that for all three areas, skin was irritated and redness was increased by more than 30% at the end of this phase. During and at the end of the subsequent treatment phase it could be shown that treatment with CefiraProtect CLR™ led to an accelerated and more effective reduction of the initial irritation as compared to the skin treated with placebo or to untreated skin. Interestingly and most importantly, at the end of the third phase of the study the remarkable effect of CefiraProtect CLR™ on the resilience of skin could be shown. The formulation containing CefiraProtect CLR™ (3%) was almost twice as efficient in making skin more resilient against the skin exposome than the identical formulation which did not contain CefiraProtect CLR™ (placebo) (see figure 10).

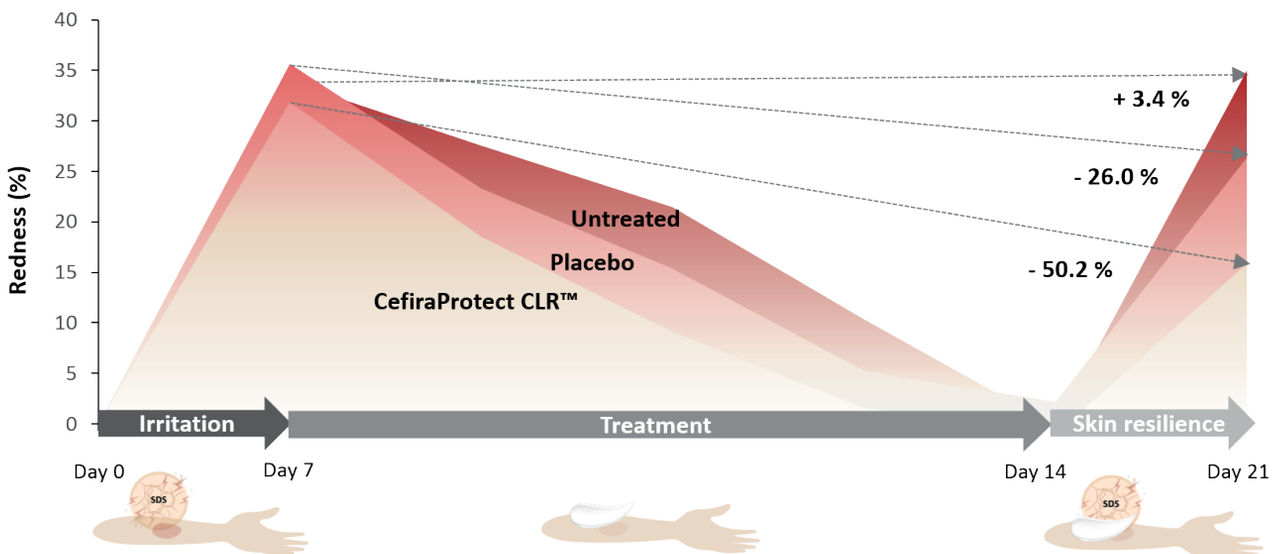


Fig. 10: Reduction of redness and resilience of skin

In vivo study 2: skin barrier function

A study was performed on the face of 13 volunteers (all female, 36–63 years old, average: 53.6 years). Test products (CefiraProtect CLR™ (3%) and corresponding placebo) were applied twice daily on the hemiface for 28 days. Skin barrier function was determined with Tewameter® TM 300 (Courage + Khazaka electronic GmbH, Germany). Barrier function at the beginning of the study was set at 0%.

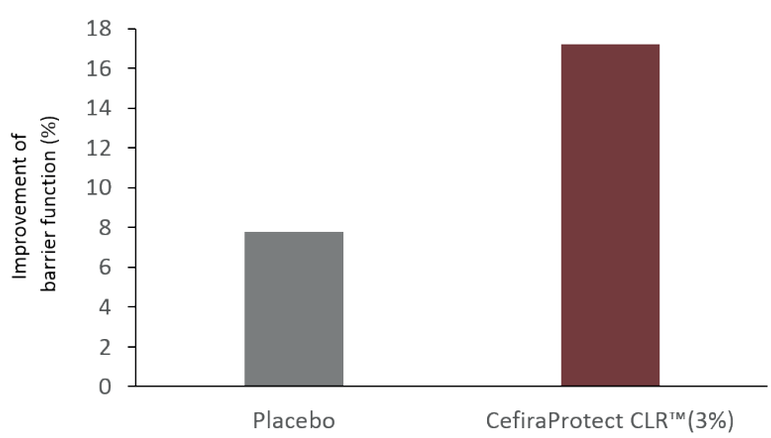


Diagram 5: Improvement of barrier function

Result: Both formulations showed to improve barrier function, but the effect of the formulation containing CefiraProtect CLR™ was more than double that of the corresponding placebo formulation (see diagram 5).

In vivo study 3: hidden inflammation

Inflammatory processes are ubiquitous in facial skin. These processes, however, are not always visible or directly perceivable, but they might still have negative outcomes for the skin in the long run. An elegant way of visualizing these processes is based on the Antera 3D technology (Miravex Limited, Ireland) which allows visualization and quantification of blood flow in the skin ("hemoglobin average level") (see figure 11). Blood flow in the skin is an important indicator of inflammatory processes.

Thirteen volunteers (7 female, 6 male) were enrolled in the study. They were 35–64 years old, average: 52.4 years. Hemoglobin average level at t = 0 of skin before treatment with CefiraProtect CLR™: >32.

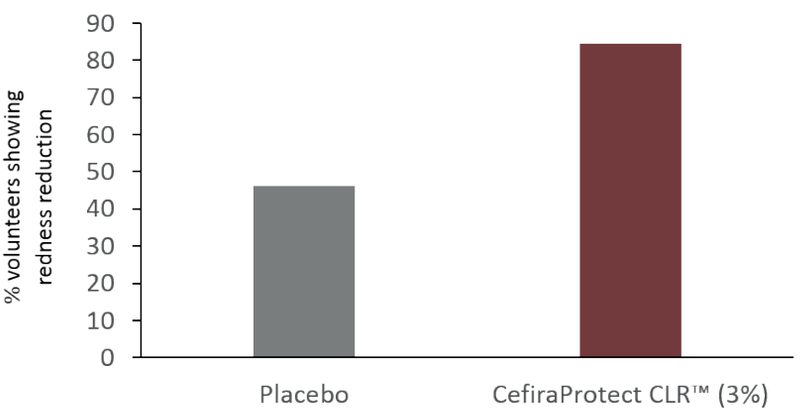


Diagram 6: Reduction of hidden inflammation

Result: The skin of only less than half of the volunteers treated with placebo showed a significant reduction of hidden inflammation, whereas CefiraProtect CLR™ reduced hidden inflammation in almost 90% of the cases (see diagram 6). As was shown in previous in vivo studies looking at skin resilience and skin barrier function, the formulation containing CefiraProtect CLR™ was almost twice as effective as the same formulation without CefiraProtect CLR™ (placebo).

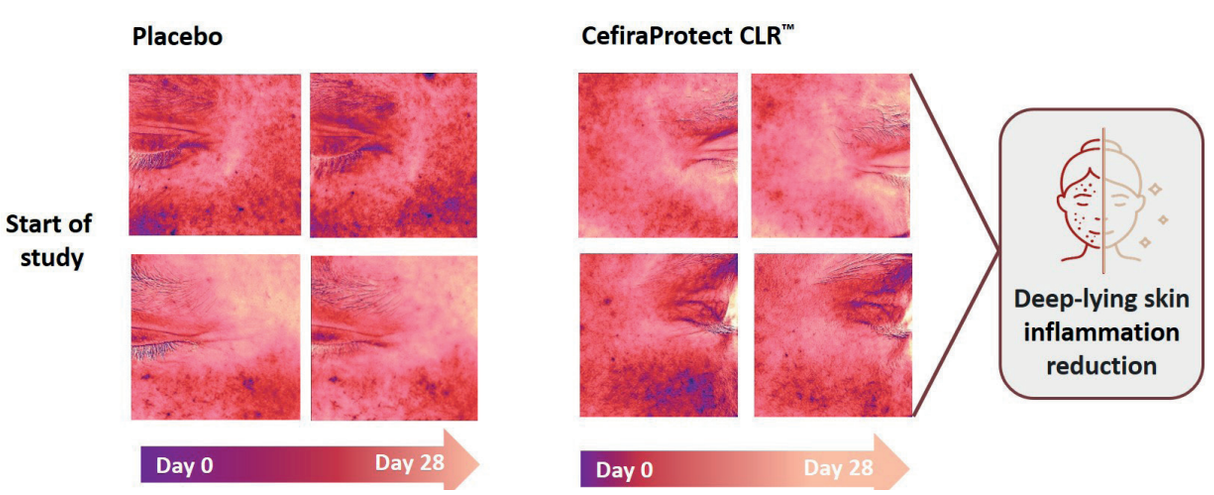


Fig 11: Reduction of hidden inflammation using Antera 3D

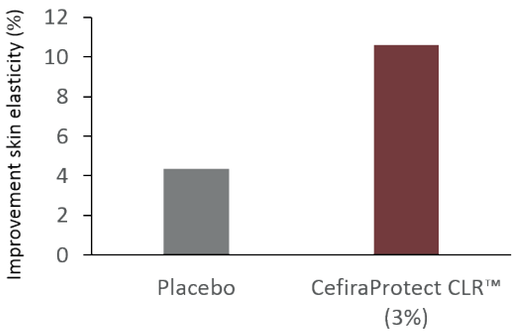


Diagram 7: Improvement in skin elasticity

In vivo study 4: skin firmness and elasticity

In maintaining full functionality, skin also constantly manages its mechanical properties. A study was performed on 16 volunteers (9 female, 7 male), 36–64 years old, average: 54.1 years. Determination of facial skin elasticity was based on R2 as obtained from measurements with a Cutometer® dual MPA 580 (Courage + Khazaka electronic GmbH, Germany). R2 at t = 0 of skin before treatment with CefiraProtect CLR™: <63. Skin elasticity at t = 0 was set at 0%.

Another study was performed on facial skin of 10 volunteers (6 female, 4 male), 35–64 years old, average: 51.5 years. Based on R0 as determined with Cutometer® dual MPA. R0 at t = 0 of skin before treatment with CefiraProtect CLR™: >0.76. Skin firmness at t = 0 was set at 0%.

Results: Facial skin elasticity (see diagramm 7), but especially firmness (see diagramm 8), were strongly increased after treatment with CefiraProtect CLR™.

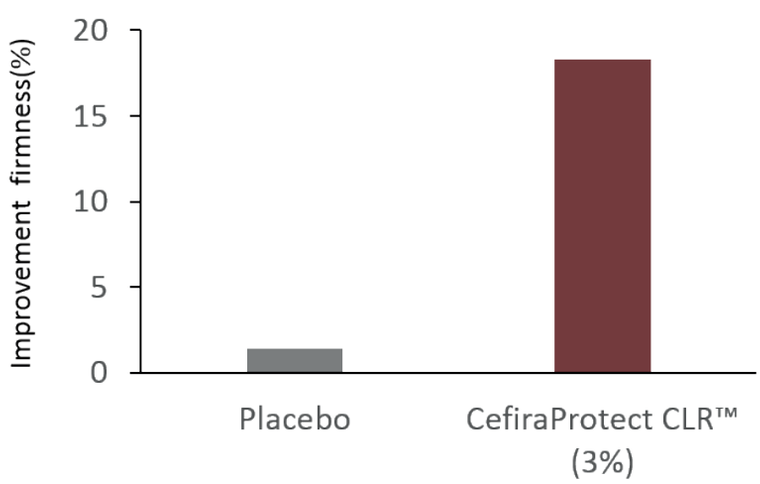


Diagram 8: Improvement in skin firmness

CONCLUSIONS

CefiraProtect CLR™ offers a promising advanced skincare solution aimed at addressing the challenges posed by the exposome on facial skin. As highlighted, the skin is continually exposed to a myriad of external stressors that contribute to oxidative stress, protein oxidation, and subsequent skin aging. Traditional antioxidants in skincare formulations often lack stability and can inadvertently exacerbate oxidative stress. CefiraProtect CLR™, inspired by the health benefits of kefir and containing the hero-ingredient kefiran, is harnessed through innovative vegan, cell-based fermentation technology and effectively supports the skin's antioxidative defenses.

Extensive in vitro, ex vivo, and in vivo studies substantiate the efficacy of CefiraProtect CLR™ in reducing free radicals, improving skin's antioxidative potential, and, importantly, minimizing protein oxidation. The studies demonstrate that CefiraProtect CLR™ not only enhances the skin's barrier function but also significantly reduces subclinical inflammation, thereby promoting a more resilient and robust skin structure. The observed improvements in skin firmness and elasticity further affirm CefiraProtect CLR™'s role in maintaining youthful and healthy skin.

As a daily skincare companion, CefiraProtect CLR™ stands out by aligning with the growing consumer demand for sustainable, vegan, and environmentally friendly products, making it a comprehensive solution for modern skincare needs. Its incorporation into facial skincare routines can provide effective protection and rejuvenation,

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