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Award

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1 | IFF Health & Biosciences

Advances in Enzyme Engineering: Delivering on the Need for Sustainable Laundry Detergents

2 | GloryActives GmbH

Protective Beauty – Holistic Skin Protection through Enzymes

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Sustainable Yeast Oil – What the Fat?

SOFW JOURNAL
BEST PAPER AWARD 2023

BERLIN, GERMANY, OCTOBER 25, 2023

For the 4th year in a row, the SOFW award was presented to the authors of the three best articles of the year published in SOFW journal. Congratulations to the winners!

At the SEPAWA® CONGRESS 2023 in Berlin, Robert Fischer presented a certificate and the SOFW award to the authors. The three contributing authors had the opportunity to give a brief overview of their award-winning article to the congress attendees.

A total of 51 articles published in SOFW journal – issues November 2022 to July/August 2023 from the Home & Personal Care and Fragrance Industries – were submitted and reviewed by a 9-member jury.

Here are the three awarded articles:

The winners of this year's SOFW award together with the editor in chief of SOFW Journal: Enda Carey (IFF Health & Bioscience), Robert Fischer (SOFW journal), Maximilian Webers (COLIPI) and Dr. Volker Krug (GloryActives) at the award ceremony.

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The winner of the first prize is the article:

1 Advances in Enzyme Engineering: Delivering on the Need for Sustainable Laundry Detergents

Authors: A.J. Hoekstra, E. Carey, T.P. Graycar
Company: IFF Health & Biosciences

Abstract: There is a market need for more sustainable laundry detergents. Consumers are looking for cleaning products that are biobased and biodegradable, but do not comprise on stain removal performance. Enzymes play a key role in today's liquid laundry detergents. This article reports on recent advances in biotechnology to improve the stability and low temperature performance of enzymes for laundry. It also demonstrates how protein engineering can enable detergent formulators to remove chemical stabilizers.

Picture Credits © Katrin Heyer

The winner of the second prize is the paper:

2 Protective Beauty – Holistic Skin Protection through Enzymes

Authors: S. Christian, V. Krug
Company: GloryActives GmbH

Abstract: Every day, our skin is exposed to many factors that promote premature skin ageing. Two key factors are also closely related to each other: UV radiation and free radicals. It is known that UV radiation alone is responsible for 80% of the visible signs of facial skin ageing [1]. Therefore, our skin needs a reliable repair and protection system. Following nature's example, enzymes are ideal components for such a system, as they offer a highly efficient and long-lasting effect. The active ingredient Glorydermal® GUARD contains a synergistically acting complex consisting mainly of two enzymes: the repair enzyme photolyase, which is derived from microalgae, and an antioxidant enzyme in the form of an iron peptide. A liposomal encapsulation of the enzymes additionally improves their penetration into the skin.
The repair enzyme photolyase repairs UV-induced DNA damage, the so-called CPDs (Cyclobutane Pyrimidine Dimers), very efficiently and faster than the body's own repair mechanisms. The antioxidant enzyme neutralises ROS (Reactive Oxygen Species) including free radicals long-lasting. Like an enzyme, it is not used up and therefore offers a long-term radical protection. Efficacy studies on human 3D full thickness skin models show the synergistic long-term repair and protection provided by these two enzymes to effectively prevent premature skin ageing.

The winning article of the third prize is:

3 Sustainable Yeast Oil – What the Fat?

Authors: J. Heuer, P. Arbter
Company: COLIPI GmbH

Abstract: After almost three years of an ongoing pandemic, climate change sent its regards this summer to remind humankind of what the future will look like. With political uncertainties arising worldwide, a sustainable oil source becomes increasingly important in both terms: ecological and economical. Yeast oil, triacylglycerides produced by oleaginous yeasts, is a promising alternative or even replacement to plant oils like cocoa butter, palm oil and similar. It has a small ecological footprint, can be produced locally in a reliable manner and has unique characteristics making it functional in multiple applications. The following article aims to give an exciting glimpse into a trending topic and COLIPI, a startup located in Hamburg dedicated to leading the fat revolution.

Advances in Enzyme Engineering: Delivering on the Need for Sustainable Laundry Detergents

A.J. Hoekstra, E. Carey, T.P. Graycar

abstract

There is a market need for more sustainable laundry detergents. Consumers are looking for cleaning products that are biobased and biodegradable, but do not comprise on stain removal performance. Enzymes play a key role in today's liquid laundry detergents. This article reports on recent advances in biotechnology to improve the stability and low temperature performance of enzymes for laundry. It also demonstrates how protein engineering can enable detergent formulators to remove chemical stabilizers.

Introduction

Enzymes have been used for many years in detergents, and their penetration globally is still growing. They are key contributors to sustainable cleaning. Enzymes are biodegradable, efficient, and enable low temperature cleaning. They are biocatalysts that can be produced by industrial fermentation under mild processing conditions. However, enzymes are also proteins, and their activity can be negatively affected by other ingredients in the detergent, especially in liquid detergents. The key to solving some of these formulation problems is protein engineering, or in other words: accelerating enzyme evolution in the lab to meet consumer needs.

Enzyme Stabilization in Liquid Detergents

A key enzyme functionality in detergents is protease. Protease hydrolyses protein and protein is part of many different stains that consumers can find on their clothes. Protease contributes to cleaning by breaking down protein in foodstuffs like meat, in bodily fluids like blood, and in outdoor stains such as grass. However, protease is also a protein. Hence, there is a risk that protease breaks down itself or other enzymes when stored in a liquid detergent for several weeks.

The conventional way of addressing this problem is to add enzyme stabilizers to the detergent. The function of these stabilizers is to slow down the reactivity of the enzyme in the liquid detergent by inhibiting the catalytic triad or active center [1]. Another approach is to reduce the water activity of the formulation.

The mechanism of enzyme activity loss can be broken down in two different steps. Unfolding of an enzyme is caused by surfactants. An enzyme can have hydrophilic and hydrophobic segments and typically has a surface charge depending on the pH of its environment. The interaction with surfactants can result into loss of the three-dimensional structure of the enzyme, also known as unfolding. This makes the enzyme susceptible to proteolysis, or hydrolysis by protease. Unfolding and consecutive proteolysis results into activity loss of enzymes during storage.

Materials and Methods

Enzyme activity in a detergent is measured by means of a biochemical assay. This assay is used to measure the impact of different detergent compositions on the stability of enzymes. The enzyme is dosed at a known quantity into the detergent which is subsequently stored in an incubator. Typically, the detergent is stored for 8 to 12 weeks at an elevated temperature to accelerate the reaction. Aliquots are taken at fixed time intervals, and these aliquots are mixed with an artificial substrate under controlled conditions. Hydrolysis of the enzyme substrate results into a color reaction, and the color intensity is directly proportional to the residual enzyme present in the sample.

In this study, the protease and mannanase activity were measured by means of a colorimetric assay. For protease, the rate of degradation of N-succinyl-ala-ala-pro-phe-p-nitroanalide, a substrate commercially available from Sigma Aldrich, was monitored. The release of the substrate's p-nitroanalide is measured at 405 nm. The assay for mannanase activity is based on the hydrolysis of a locust bean gum substrate (Sigma Aldrich). The amount of reducing sugars generated during the reaction was measured at 560 nm using Pierce™ BCA Protein Assay reagent from Thermo Scientific. Colorimetric analysis was done on a Gallery auto-analyzer from Thermo Scientific and SpectraMax® ABS Plus from Molecular Devices.

The cleaning performance of the heavy-duty liquid (HDL) detergent was measured by using an enzyme responsive stain set that is commercially available from Center for Testmaterials (CFT BV, The Netherlands). Experiments were carried out in Miele W1935 WPS Ecoline (Cotton, Short cycle) at 30°C and water hardness of 14°GH using a 3.0 kg cotton wash load and 4 strips SBL2004 soil ballast. The detergent dosage was 35 ml/wash. Stain removal measurements were carried out using a Mach5^{+} multi area spectral imaging device (CFT BV/Colour Consult), averaged using 2 internal and 4 external replicates, and expressed in a Stain Removal Index (SRI) % based on Delta E color differences.

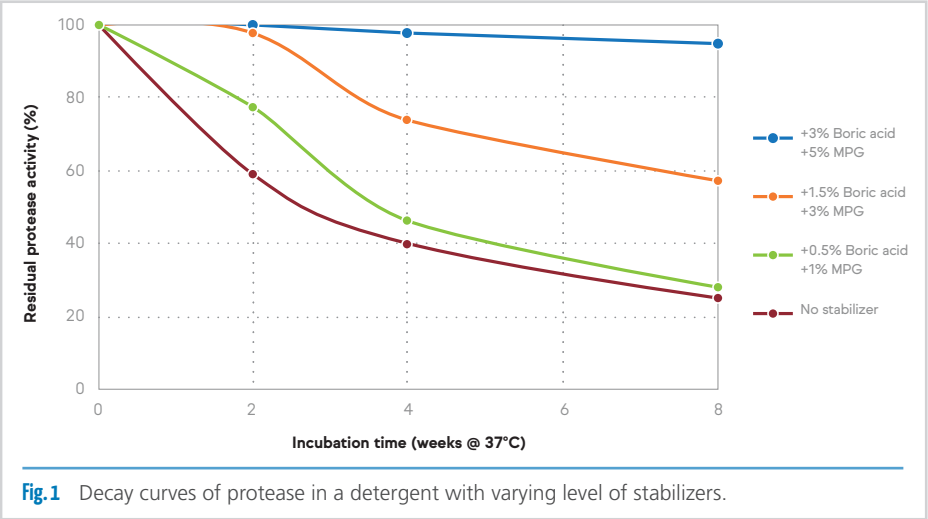

Fig. 1 Decay curves of protease in a detergent with varying level of stabilizers.

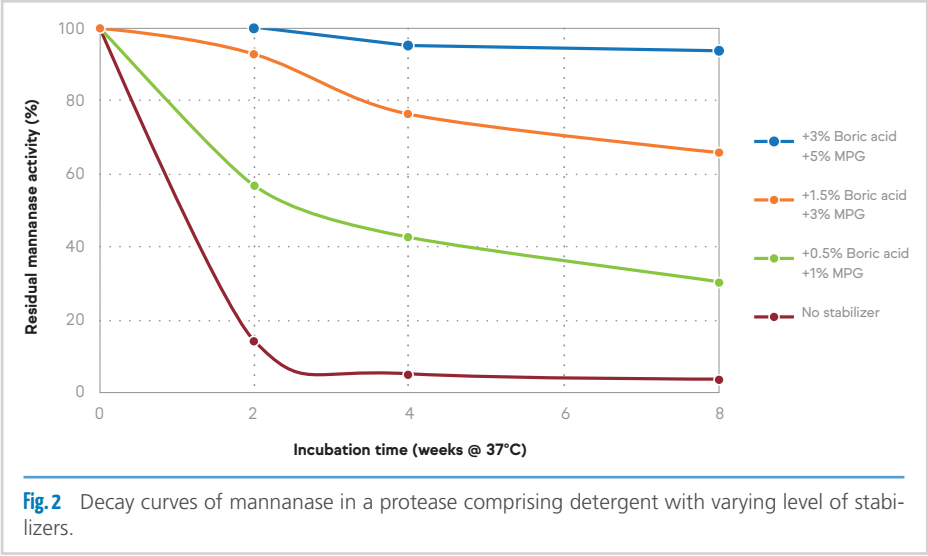

Fig. 2 Decay curves of mannanase in a protease comprising detergent with varying level of stabilizers.

Figure 1 shows the decay curve for a protease in different detergents during storage at 37°C. On the horizontal axis the incubation time is given; the vertical axis shows the residual protease activity as measured by a biochemical assay. Four different decay curves are shown which represent detergent compositions with varying levels of enzyme stabilizers. The figure demonstrates that by increasing the boric acid and propylene glycol level in the formulation, the protease stability can be improved significantly.

Stabilizers are also effective against proteolysis, or the breakdown of other enzymes by protease. This is demonstrated in **Figure 2**, where the decay curve for mannanase is presented in a detergent with varying level of chemical stabilizers. Without stabilizers the mannanase loses its activity rapidly because of unfolding and proteolysis. The addition of boric acid and propylene glycol to the detergent has a dramatic effect on the stability of mannanase as well. It demonstrates that the protease active center is effectively inhibited by boric acid and propylene glycol.

The Issue with Chemical Stabilizers

The addition of boric acid to a liquid detergent is effective in stabilizing enzymes, however, there are several reasons to reconsider this approach.

First, consumers are becoming increasingly conscious about their environmental footprint. Millions of consumers around the globe do their laundry every day resulting in large quantities of water and chemicals used in the laundry process. Consumers are looking for products that address their concerns about sustainable cleaning. We see brands increasingly communicating their credentials to the consumer by making environmental, natural, and functional claims on the product pack.

Another important factor is legislation in Europe. According to the Classification, Labelling and Packaging Regulation, or CLP, boric acid and related boron compounds are classified as reprotoxic 1B. A recent CLP amendment will further restrict the use of boric acid by applying the generic concentration limit of 0.3% by weight in the formulation. This limits the efficacy of boric acid as a protease inhibitor, and the industry is looking for alternative ways to stabilize their enzymes in liquid detergents. There are options to improve enzyme stability by product concentration and reduction of water activity, for example in unit dose detergents, or to formulate with additional calcium or formate. However, calcium chloride and sodium formate are generally less effective as a stabilizer compared to boric acid.

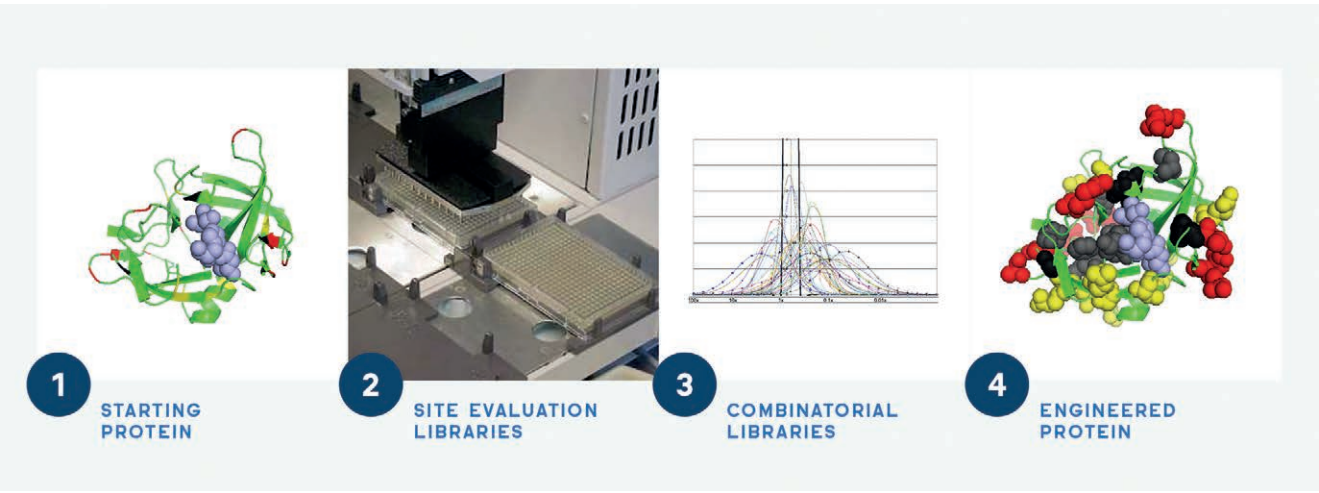

Fig. 3 Schematic representation of the protein engineering approach for enzymes [2].

Another complicating factor is the recent price increases for solvents such as propylene glycol and glycerol. These solvents are effective in reducing the water activity of the formulation. Rising energy prices and supply chain disruptions have forced detergent formulators to reduce the use of these solvents, which puts the stability of enzymes at risk. In summary, there is a need for another approach to enzyme stabilization in liquid detergents.

The Protein Engineering Approach

Protein engineering enables the improvement of specific enzyme properties by changing the amino acid sequence. Specific amino acid mutations can result in better cleaning performance on specific stains and/or lower temperature, and improved enzyme stability in a detergent.

A schematic representation of the protein engineering approach is shown in **Figure 3**. The first step in the process is to build site evaluation libraries in which each residue of the starting protein is mutated with all nineteen possible amino acid substitutions at that site. These libraries of single mutation variants are then tested for commercially relevant properties such as detergent stability and stain cleaning performance. Mutations found to improve at least one property and not impair any other property are then selected for use in creating libraries of modified enzymes having two or more mutations in combination. Product candidates are identified through successive rounds of combinatorial library construction, testing, and data analysis.

An example of the recent advances in protein engineering for liquid protease is shown in **Figure 4**. The protease is dosed into a liquid detergent that contains no enzyme stabilizer like boric acid. PREFERENZ® P 100 has not been engineered for stability and loses its activity rapidly without stabilizers. Protein engineering has improved the stability of PREFERENZ® P 300 significantly, although a low level of stabilizers is still required in this detergent [3]. Our next generation protease for liquid detergents, PREFERENZ® P 400, delivers a further step change improvement in stability. After 8 weeks of incubation at 37°C even 70% of protease activity remains in this detergent without using chemical stabilizers.

Protein engineering allows us to improve the stability of secondary enzymes as well. **Figure 5** shows the level of improvement that can be achieved by improving the inherent stability of a mannanase when combined with protease in a detergent without stabilizers. PREFERENZ® M 200 mannanase has been engineered for stability in liquid detergents containing protease. By combining PREFERENZ® P 400 protease and PREFERENZ® M 200 mannanase a nearly perfect stability of the

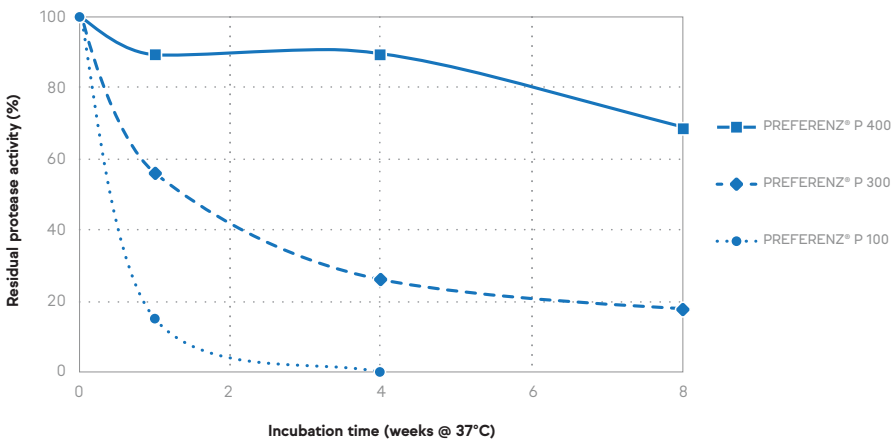

Fig. 4 The effect of protein engineering on protease stability in liquid laundry detergent.

mannanase in this detergent can be achieved during an accelerated storage test. It illustrates the potential of using the protein engineering approach for liquid protease as well as secondary enzymes like mannanase.

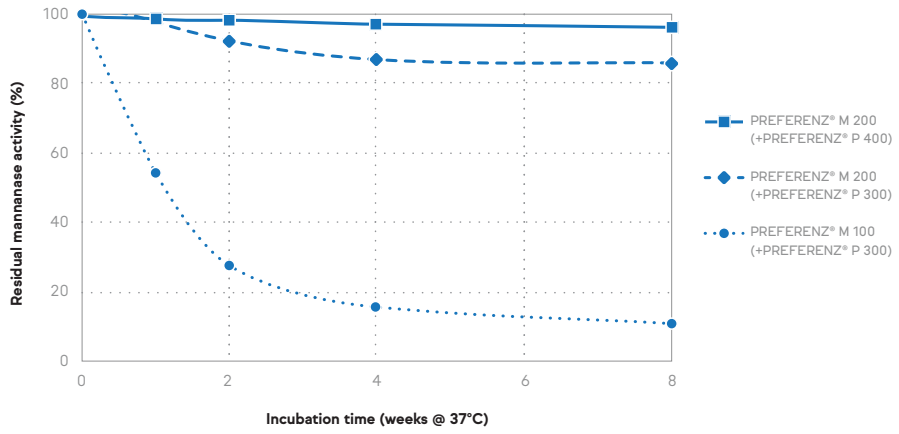

Fig. 5 Stability profile for different combinations of protease and mannanase in a liquid detergent.

Enabling Low Temperature Cleaning

One of the key attributes of a detergent is its stain removal performance. In addition to improving enzyme stability, protein engineering allows for the development of enzymes that are more effective at ambient wash temperatures. Consumers are incentivized by the rising cost of energy to select a low temperature wash cycle on their washing machine, but not at the expense of a poorer cleaning result. **Figure 6** shows the level of stain removal improvement achieved for the next generation protease PREFERENZ® P 400 during a cold wash (20°C) in a European front-loading automatic washing machine. The boost in cleaning performance is clearly seen over the broad range of protease substrates found in everyday consumer stains such blood, chocolate, egg, milk, and grass. This latest protein engineering development enables improved cleaning performance at low temperature and thus supports sustainable cleaning.

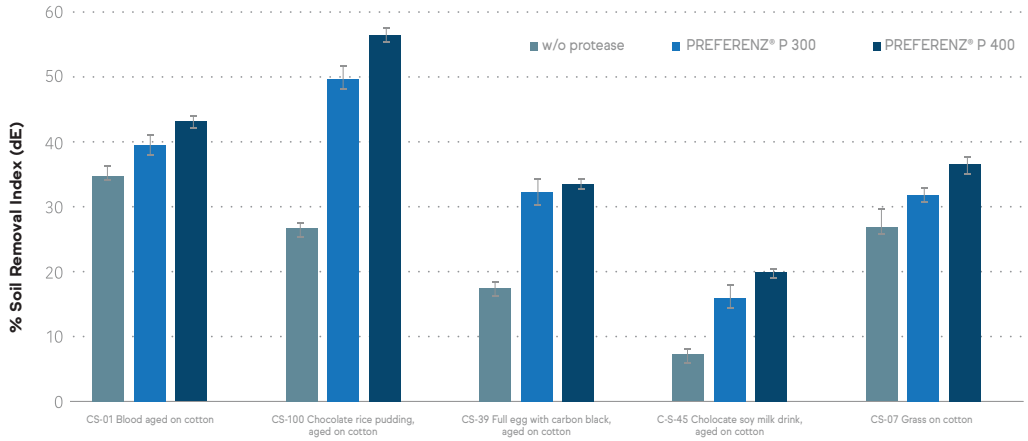

Fig. 6 Cold wash (20°C) cleaning performance of protease under typical European washing conditions.

Conclusions and outlook

The latest advances in protein engineering demonstrate how we can address the consumers' need for sustainable laundry detergents. Protein engineering enables the removal of chemical stabilizers from the detergent formulation. By developing inherently stable enzymes there is no need to formulate using a pre-stabilized protease. Also, the next generation of IFF enzymes provides a robust cleaning performance, fresh and after storage, even at low wash temperatures.

At IFF we are passionate about the potential of protein engineering and industrial biotechnology, and we continue to develop new biomaterials that deliver performance, naturally better.

Acknowledgments:
Dr. Sina Pricelius (IFF Health & Biosciences) is greatly acknowledged for a critical review of the article and correction of the German translation.

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Protective Beauty – Holistic Skin Protection through Enzymes

S. Christian, V. Krug

Every day, our skin is exposed to many factors that promote premature skin ageing. Two key factors are also closely related to each other: UV radiation and free radicals. It is known that UV radiation alone is responsible for 80% of the visible signs of facial skin ageing [1]. Therefore, our skin needs a reliable repair and protection system.
Following nature's example, enzymes are ideal components for such a system, as they offer a highly efficient and long-lasting effect. The active ingredient Glorydermal® GUARD contains a synergistically acting complex consisting mainly of two enzymes: the repair enzyme photolyase, which is derived from microalgae, and an antioxidant enzyme in the form of an iron peptide. A liposomal encapsulation of the enzymes additionally improves their penetration into the skin.
The repair enzyme photolyase repairs UV-induced DNA damage, the so-called CPDs (Cyclobutane Pyrimidine Dimers), very efficiently and faster than the body's own repair mechanisms. The antioxidant enzyme neutralises ROS (Reactive Oxygen Species) including free radicals long-lasting. Like an enzyme, it is not used up and therefore offers a long-term radical protection. Efficacy studies on human 3D full thickness skin models show the synergistic long-term repair and protection provided by these two enzymes to effectively prevent premature skin ageing.

Introduction

Skin protection is not a question of age. With a total surface area of about two square metres, our skin is our largest organ that covers and protects our body. It is as unique as it is diverse and accompanies us throughout our lives – so it is worth taking good care and protecting it **(Figure 1)**.

In our daily lives, our skin is exposed to many influences that can be damaging. One of these influences is UV radiation. This is also associated with free radicals, which can also affect our skin and let it age more quickly. But UV radiation and free radicals are not influences that can just be reduced to the summer months. Both accompany our daily lives, sometimes to a greater or lesser extent, but also whenever we are not directly aware of them. As the main causes of premature skin ageing, UV radiation and free radicals can lead to signs of ageing such as photoageing and wrinkles through DNA damage and oxidation processes.

The active ingredient Glorydermal® GUARD targets these two influencing factors centrally and offers a reliable repair and protection system that protects our skin like an invisible shield against the negative effects of UV radiation and free radicals. It repairs UV-damaged DNA and neutralises free radicals synergistically and long-lasting.

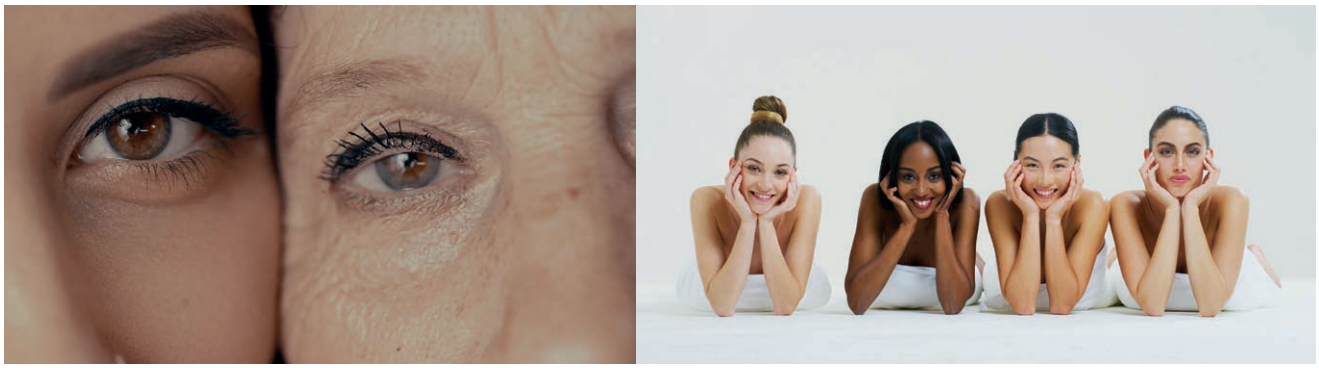


Fig. 1 Our skin is more than just the shell of our body. Its care and protection should therefore be essential elements of modern beauty concepts. This basis is comprehensively taken up by the trend Protective Beauty.

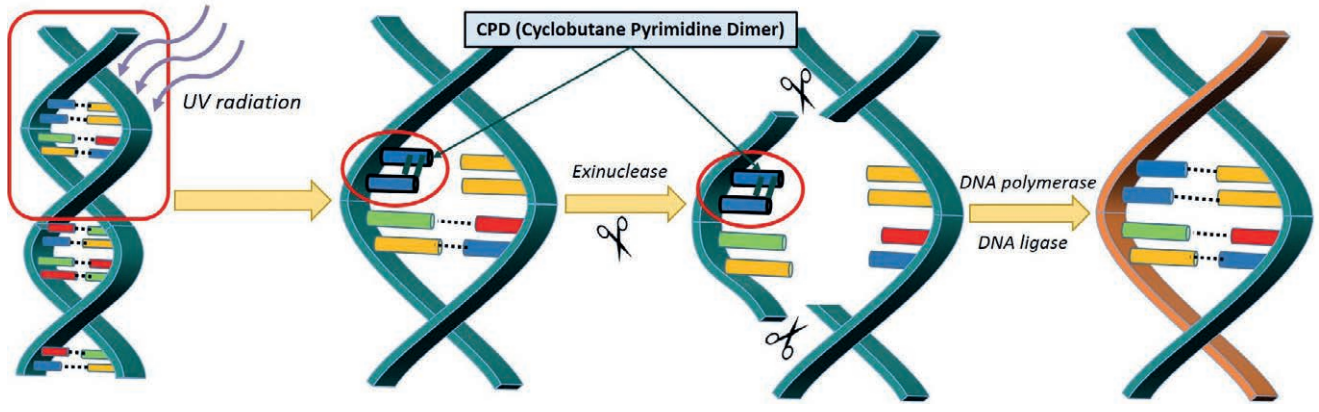


Fig. 2 Human endogenous repair mechanism of UV-damaged DNA (nucleotide excision repair) to remove CPDs, delineated according to the research of Lindahl et al. which was awarded the Nobel Prize in Chemistry in 2015 [2]. Repair via an intermediate step in which the CPDs are generously separated and the DNA strand is subsequently re-synthesised.

Enzymes are the conductors of life – Efficacy based on the example of nature

The research for suitable active components yields numerous active ingredients, especially in the area of free radical neutralisation, which are used as antioxidants in cosmetic products. However, in DNA repair and the neutralisation of free radicals, effectiveness alone is not the decisive factor; speed and a long-term effect are also important to ensure that our skin can be effectively protected throughout the day.

UV radiation and free radicals are both very well-known, natural influences of ancient origin. Since the beginning of life, organisms depend on counteracting these influences with effective repair and protective mechanisms in order to survive. Enzymes play an essential role in these processes. They enable and accelerate many biochemical reactions and have important functions in the metabolism of organisms. A crucial feature is also that enzymes are not used up even over many reaction cycles and thus uniquely combine a particularly fast and long-lasting effect.

Therefore, enzymes provide the optimal basis for fulfilling the above-mentioned important requirements for effective skin protection against the negative effects of UV radiation and free radicals. The synergistic active ingredient complex of Glorydermal® GUARD is thus composed mainly of two enzymes. It combines the DNA repair enzyme photolyase, which is extracted from microalgae, with an antioxidant enzyme in the form of an iron peptide for neutralising free radicals. Both enzymes are also liposomally encapsulated to improve skin penetration.

Enzymes can support the repair mechanisms of our skin very effectively. The repair enzyme photolyase from microalgae, for example, offers a 10 to 100 times faster repair of UV-damaged DNA than the body's own human repair mechanisms. The origin of this highly efficient repair lies in the evolution of microalgae: billions of years ago, they already developed an effective protection and repair system against intense UV radiation, which was significantly stronger in this epoch than it is today, as it was not diminished by an atmospheric layer. This layer developed only later during the course of the earth's history - but the repair and protective mechanisms of the microalgae have been preserved, so that they can be made available to cosmetics today.

The most common UV-induced lesion of DNA is Cyclobutane Pyrimidine Dimers (CPDs), in which UV radiation separates opposite base pairings in the DNA double helix and two adjacent bases of the same strand subsequently combine incorrectly. The human endogenous repair mechanism to eliminate these DNA damages involves an intermediate step in which the CPDs with the affected strand segment are generously separated **(Figure 2)**. The complementary DNA strand is finally re-synthesised.

The body's own repair process can be accelerated considerably with the help of the repair enzyme photolyase, since photolyase can repair the DNA damage directly without an intermediate step by cleaving the CPD bonds in just one step **(Figure 3)**. Photolyase is activated by light and is particularly active in the range of blue radiation. Thus, it can be used directly during sun exposure, for example, to repair UV-induced damage of the DNA, whereas this never replaces the use of a UV filter as the primary sun protection. In combination, however, photolyase offers an extended protection concept.

With the combination of the repair enzyme photolyase and the antioxidant enzyme in the form of an iron peptide, which is designed like an enzyme that does not use itself up over many reaction cycles, the active ingredient Glorydermal® GUARD is based on a pairing of continuously working active accelerators. Like this, long-term radical protection and highly efficient DNA repair are not only ideally combined, they also show a synergistic effect.

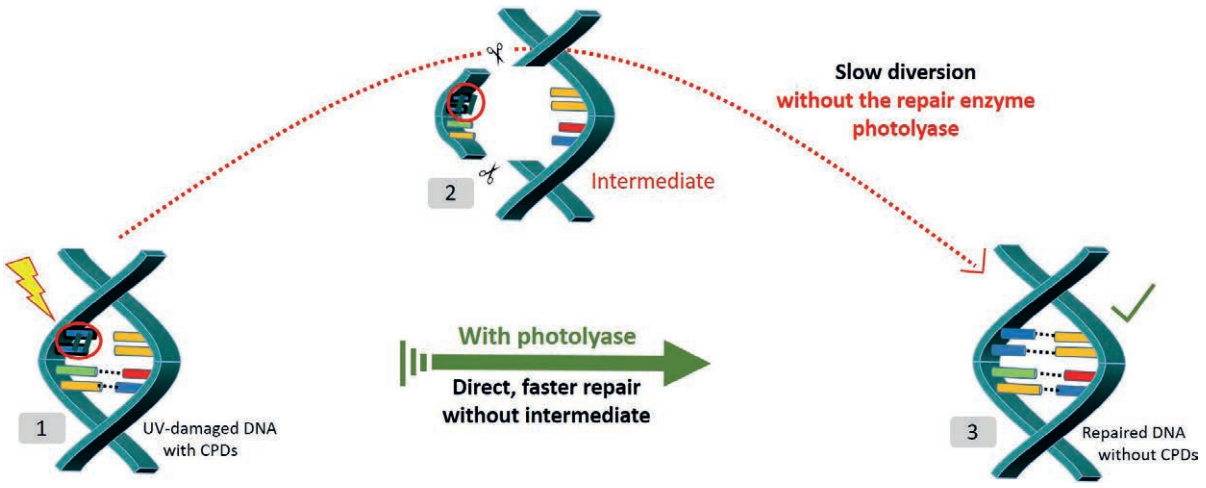


Fig. 3 Repair mechanism for the removal of CPDs with the repair enzyme photolyase. Compared to the body's own repair mechanism (dashed path above), this takes place without an intermediate directly by cleaving the CPD bonds in just one step.

Methods and results

The efficacy proof of DNA repair and long-term radical protection were gained in studies on human 3D full thickness skin models (Phenion® models [3]). Since for both effects the skin would first have to be damaged in order to be able to demonstrate both the subsequent repair of the DNA and the reduction of free radicals, no *in vivo* studies were deliberately carried out here for ethical reasons. The 3D full thickness skin models also have the advantage that they are very robust in terms of feasible study designs and also provide high reproducibility [4]. Thus, the degree of damage could be widely exploited to investigate the potential of Glorydermal® GUARD. This is particularly evident in the studies on the reduction of free radicals, in which repeated applications of a hydrogen peroxide solution (H_2O_2) with a respective exposure time of 1 h could be used (**Figure 5** and **Figure 6**) – a design that would not be possible in an *in vivo* study.

Efficacy study CPD reduction

To study the DNA repair, the reduction of UV-induced CPDs was analysed. For this purpose, the human 3D full thickness skin models were treated with an aqueous active ingredient solution and were then UV-irradiated.

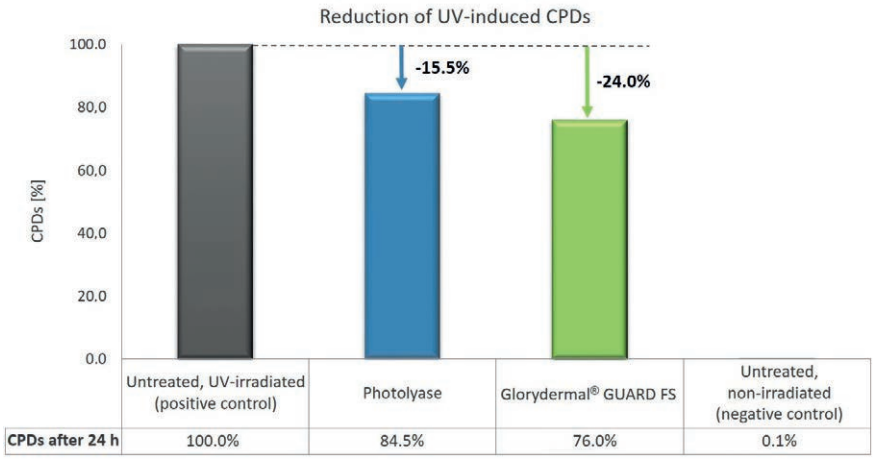


Fig. 4 Study design: human 3D full thickness skin models, used formulation: aqueous solution with 1% Glorydermal® GUARD FS or only with the corresponding photolyase concentration. Subsequent irradiation: UVB radiation (220 mJ/cm²), incubation for 24 h. Untreated, UV-irradiated = positive control (normalisation to 100%, maximum stress); untreated, not irradiated = negative control. Analysis: CPD ELISA assay (epidermal keratinocytes), % values in relation to positive control ($p<0.01$).

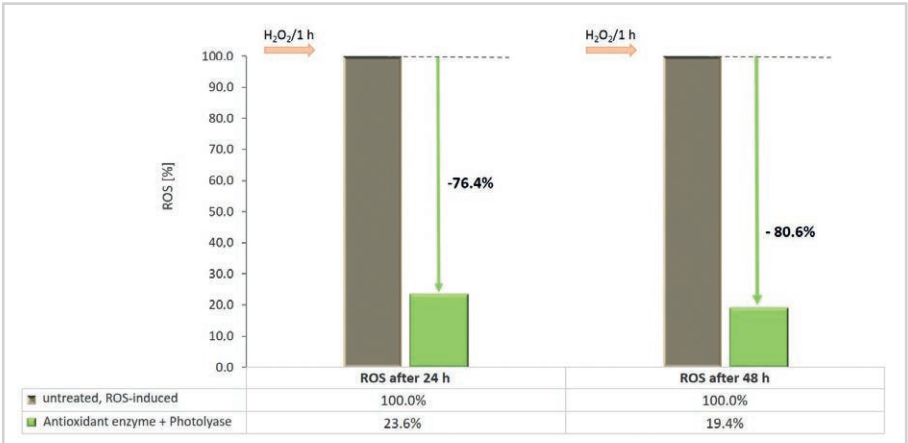
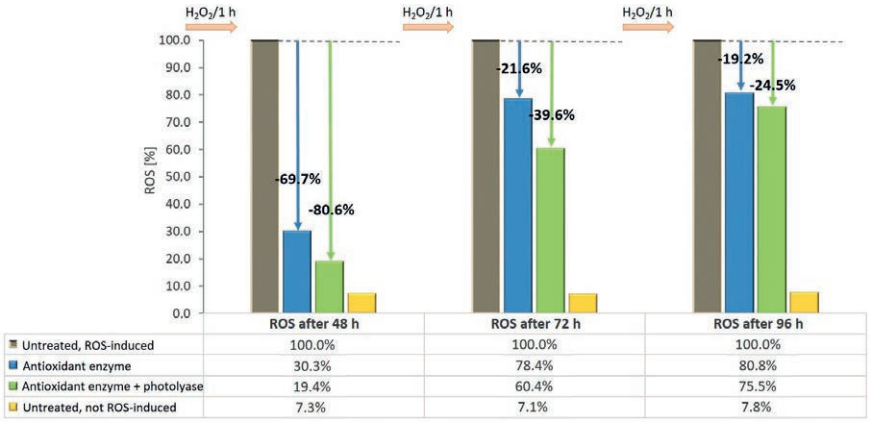


Fig. 5 Study design: human 3D full thickness skin models, used formulation: aqueous solution with 1% Glorydermal® GUARD FS with regard to the antioxidant enzyme. ROS are induced in each case by H_2O_2 treatment (treatment duration: 1 h). Untreated, ROS-induced = positive control (normalised to 100%, maximum stress). % values in relation to the positive control ($p<0.0001$).

The evaluation was performed after 24 h incubation against positive control by CPD ELISA assay on epidermal keratinocytes **(Figure 4)**. The measurement results show a significant reduction of UV-induced CPDs as well as the synergistic effect of the two enzymes. The antioxidant enzyme protects the repair enzyme photolyase from oxidative degradation, so that the latter can repair more CPDs in the same period of time than without the antioxidant enzyme. Through this synergism, the already very efficient effect of photolyase can be further increased.



	ROS after 48 h	ROS after 72 h	ROS after 96 h
Untreated, ROS-induced	100.0%	100.0%	100.0%
Antioxidant enzyme	30.3%	78.4%	80.8%
Antioxidant enzyme + photolyase	19.4%	60.4%	75.5%
Untreated, not ROS-induced	7.3%	7.1%	7.8%

Fig. 6 Study design: human 3D full thickness skin models, used formulation: aqueous solution with 1% Glorydermal® GUARD FS with regard to the antioxidant enzyme or only with the corresponding concentration of the antioxidant enzyme. ROS are induced in each case by H_2O_2 treatment (treatment time: 1 h). Untreated, ROS-induced = positive control (normalisation to 100%, maximum stress); untreated, not ROS-induced = intrinsic cell stress. % values in relation to the positive control ($p<0.0001$).

Efficacy study long-term radical protection

In this study, hydrogen peroxide solution was used as an initiator for the generation of free radicals respectively ROS (Reactive Oxygen Species) to investigate the long-term radical protection. The full thickness skin models were treated once with the active ingredient solution followed by a treatment with a hydrogen peroxide solution (exposure time of 1 h). The treatment with hydrogen peroxide was repeated every 24 h with fresh solution over an examination period of 96 h. Within 24 h after application of the active ingredient solution and initial treatment with hydrogen peroxide solution, ROS could be reduced by over 76% and after 48 h and second treatment with fresh hydrogen peroxide solution even by more than 80% **(Figure 5)**.

The reduction of ROS can be detected significantly even after 72 and 96 h accordingly, whereby the degree of reduction decreases with time, as the antioxidant enzyme in the skin is degraded over this long period of time **(Figure 6)**. As in the CPD reduction study, a synergistic effect of the repair enzyme photolyase with the antioxidant enzyme was demonstrated, shown in the same figure. This bidirectional synergism represents a significant performance advantage compared to the single enzymes. The respective enzyme concentrations in Glorydermal® GUARD are also optimally harmonised with each other regarding these synergistic effects.

Discussion

As the studies described herein show, the repair enzyme photolyase and the antioxidant enzyme (iron peptide) represent continuously acting partners and thus provide the direct response to UV radiation and free radicals, which are also closely related as key causes of premature skin ageing. The proven synergistic long-term repair of UV-damaged DNA and the also synergistic long-term radical protection are based on the enzymatic action that enzymes do not use up themselves. They are regenerated after their action is completed and are therefore available for a further reaction cycle. On this basis, the antioxidant enzyme was developed, which is a synthetic component in contrast to the repair enzyme photolyase. The reduction of ROS proven over a very long period of time demonstrates the enzymatic action of the iron peptide. In contrast to common antioxidants, which become inactive after their action, i.e. after neutralisation of free radicals, the antioxidant enzyme can undergo several reaction cycles through self-regeneration until it is naturally degraded by skin-physiological metabolic processes. The same applies to the repair enzyme photolyase.

This advantage of the enzymatic action can be explained particularly well with the antioxidant enzyme in comparison to common antioxidants **(Figure 7)**.

The effect of free radicals is, simplified, the drive for paired electrons. In contrast to molecules with paired electrons, free radicals have unpaired electrons. They compensate for this electron gap by taking electrons from other molecules, which can be components of skin cells, for example. This again creates an electron gap in these molecules, which corresponds to a damage **(Figure 7A)**.

Antioxidants have flexible electrons that they can donate to free radicals in order to neutralise them. Antioxidants can balance the resulting electron gap well, e.g. through their structure. However, they are used up in this state and can no longer donate any more electrons to free radicals for neutralisation. In return, the other molecule remains unaffected and no damage occurs, for example, to components of skin cells **(Figure 7B)**.

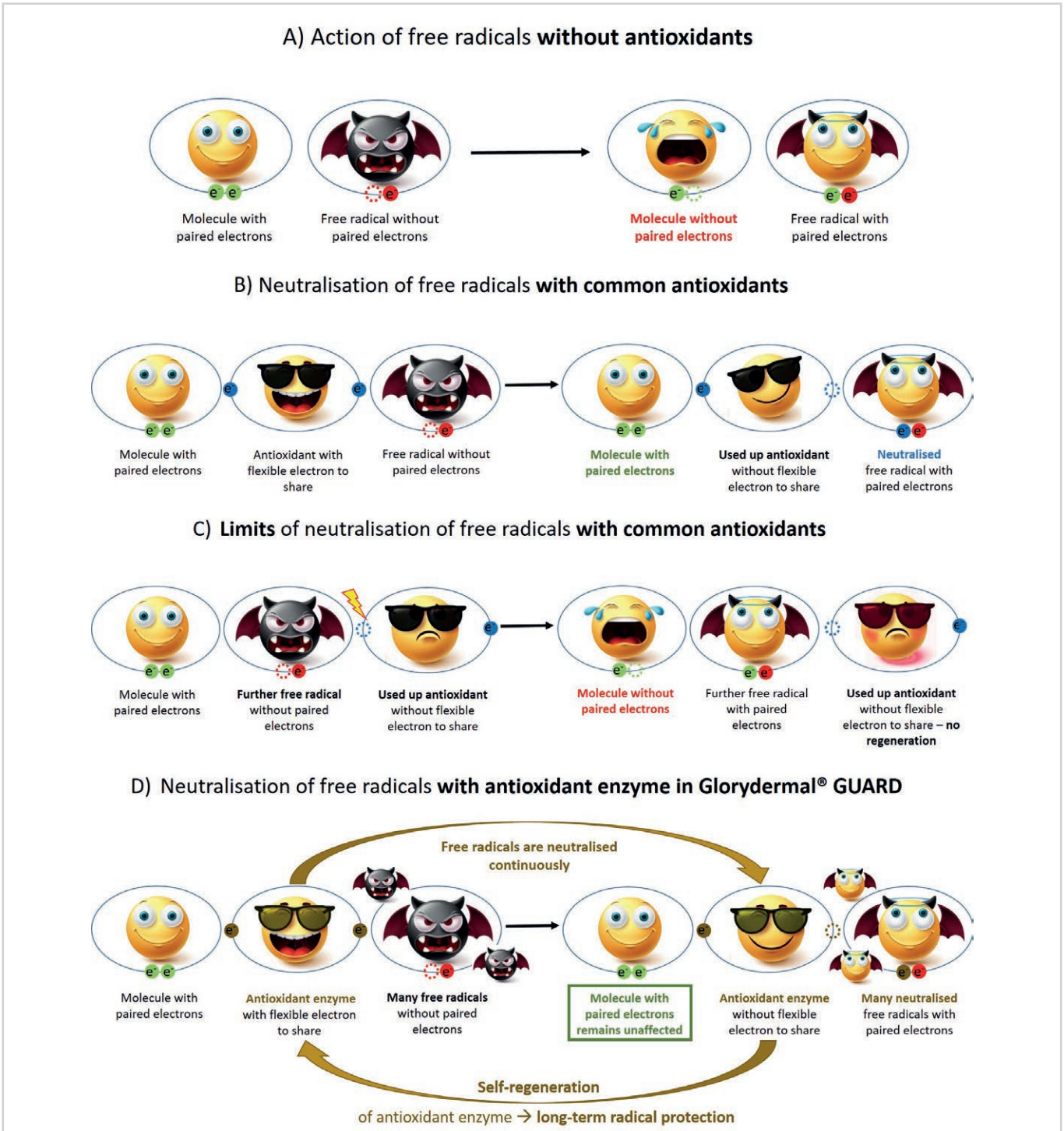


Fig. 7 A) - D) Illustration of the enzymatic action of the antioxidant enzyme (iron peptide) in the active ingredient Glorydermal® GUARD in comparison to common antioxidants.

Since used-up antioxidants cannot neutralise further free radicals due to electrons already given off, their protective effect is temporarily limited. As a consequence, like in the case without antioxidants, damage can occur to cell components that the used-up antioxidant cannot block **(Figure 7C)**.

The antioxidant enzyme in Glorydermal® GUARD targets precisely this point: it neutralises free radicals like ordinary antioxidants, but is subsequently able to regenerate itself. Like an enzyme, it does not consume itself and can therefore continuously neutralise many more free radicals. Through this long-term radical protection, skin cell components remain protected from oxidative damage in the long term **(Figure 7D)**.

The iron in the centre of the antioxidant enzyme does not chemically pass through the usual +II/+III oxidation stages for iron, but +III/+IV. The mechanism of action corresponds to that of a natural peroxidase **(Figure 8)**.

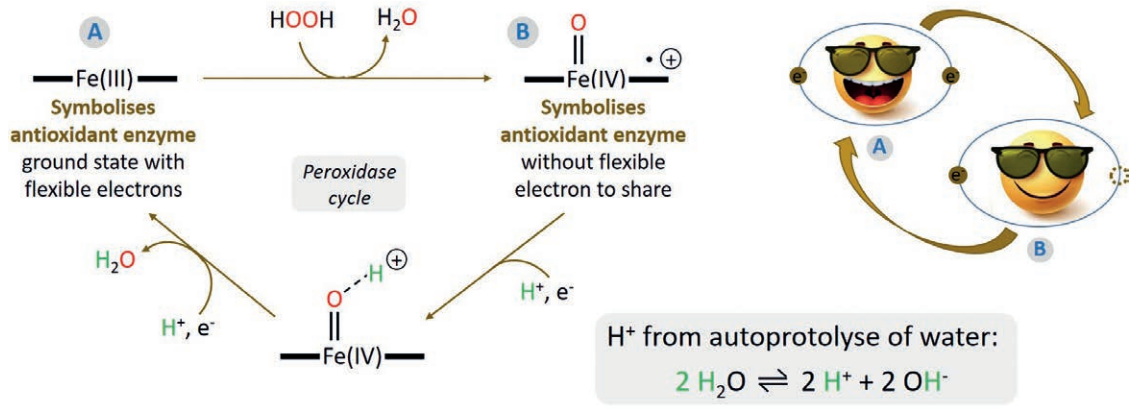


Fig. 8 Self-regeneration of the antioxidant enzyme, derived from the peroxidase cycle of horseradish peroxidase [5]. The protons required in the process are available in the medium from the autoprotolysis of water.

This holistic approach shows that the active ingredient Glorydermal® GUARD can be used in a wide range of cosmetic formulations, from daily face and body care to sun protection and after sun products.

Outlook

For the reasons described above, the proof of efficacy, which directly demonstrates both the repair of UV-damaged DNA and the reduction of free radicals/ROS, was provided via human 3D full thickness skin models and deliberately not via *in vivo* studies. However, the latter are currently being carried out to prove the resulting effects, such as wrinkle depth reduction and moisture retention, in order to complement the proof of concept already presented.

Conclusion

Premature skin ageing is primarily due to two key factors that are closely related to each other, namely UV radiation and free radicals. In the research for suitable active components slowing down premature ageing, speed and a long-term effect were essential requirements to ensure efficient repair of UV-damaged DNA and neutralisation of free radicals. Following nature's example, enzymes were therefore chosen for the active ingredient Glorydermal® GUARD to fulfil these two important demands. The repair enzyme photolyase from microalgae for repairing UV-damaged DNA (reduction of UV-induced CPDs) in combination with an antioxidant enzyme (iron peptide) in liposomal encapsulation mainly forms the synergistic active complex of Glorydermal® GUARD. This has been proven to provide comprehensive skin protection, acting like an invisible shield to protect the skin from the negative effects of UV radiation and free radicals. Due to the enzymatic mode of action, a long-term effect is also possible through continuous repair of DNA damage and long-lasting neutralisation of ROS/free radicals, which significantly exceeds the duration of action of common antioxidants. Simple incorporation in the active ingredient phase at the end of production also enables uncomplicated handling of the active ingredient in the production of cosmetic formulations, such as day care, body care, sun protection and after sun products.

The currently popular trend Protective Beauty could even become more than a trend with active ingredients like Glorydermal® GUARD. It could be the beginning of a new, holistic care concept for our skin – however, trends are fleeting, but the care and protection of our skin will remain.

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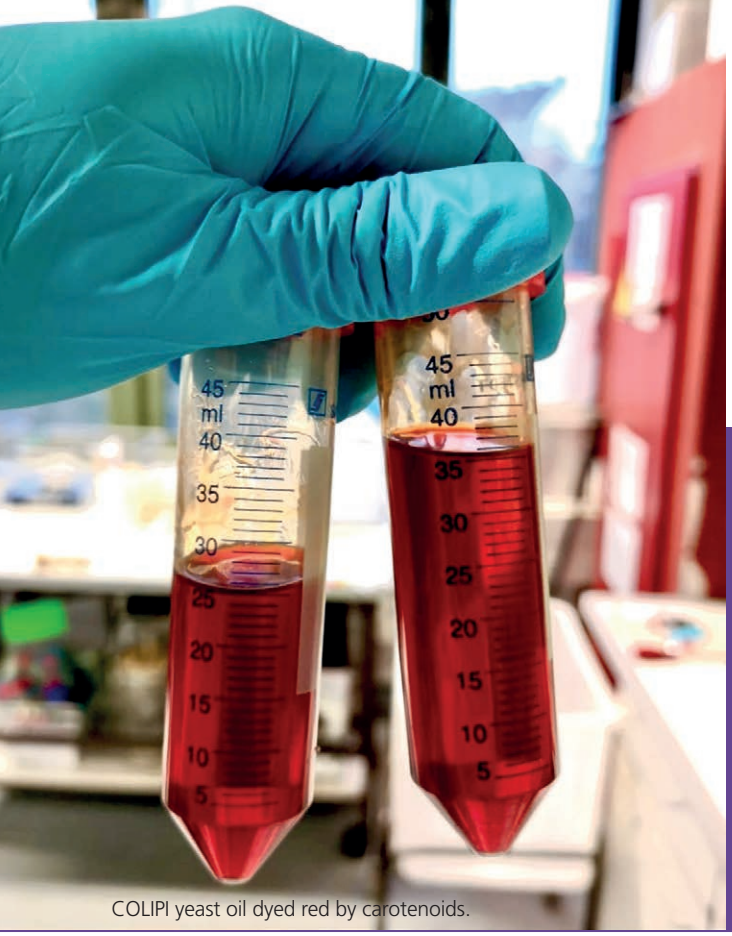

COLIPI yeast oil dyed red by carotenoids.

Sustainable Yeast Oil – What the Fat?

J. Heuer, P. Arbter

After almost three years of an ongoing pandemic, climate change sent its regards this summer to remind humankind of what the future will look like. With political uncertainties arising worldwide, a sustainable oil source becomes increasingly important in both terms: ecological and economical. Yeast oil, triacylglycerides produced by oleaginous yeasts, is a promising alternative or even replacement to plant oils like cocoa butter, palm oil and similar. It has a small ecological footprint, can be produced locally in a reliable manner and has unique characteristics making it functional in multiple applications. The following article aims to give an exciting glimpse into a trending topic and COLIPI, a startup located in Hamburg dedicated to leading the fat revolution.

Introduction and current trends

Yeasts are remarkable microorganisms. Not only are they used to produce beer, wine, bread and other products for thousands of years but some wildtype strains can store energy in the form of lipid bodies excessively. Up to 70% of the cell dry weight (CDW) of the yeast cells can consist of lipids at the end of a fermentation. Chemically, the lipids are triacylglycerides (TAGs) comparable to those of plant oils. Physically, the properties of the oils depend a lot on the composition of those TAGs, more precisely on their fatty acids. The fatty acids synthesized by the yeast mainly cover palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linolic acid (C18:2). Therefore, they appear to be a suitable alternative to universally used oils.

Speaking about the applications, there are roughly two ways of using (plant) oil in the cosmetic industry. The greatest part is not formulated as TAGs but split into glycerin, the most used cosmetic ingredient after water, and fatty acids. The fatty acids are esterified to various emollients, emulsifiers, lubricants, plasticizers and more. A smaller fraction is indeed formulated directly into products as oil. In this case, they are mostly called "effect oil" since they are not used as a structural ingredient like the fatty acid ester mentioned before but as a valuable component with beneficial properties for health, beauty or well-being. In both regards, yeast oil could replace conventional plant oils.

In the category of effect oil, a more suitable label could be "climatic effect oil". The tremendous impact of rainforest turned into monocultural farms is well known and documented. It is out of question that the sheer mass of soybean or palm oil cannot be replaced within a short time but taking the growing population into account, the first goal must be the termination of all ongoing deforestation. Administrations and institutions tighten measures to transform industries towards a more sustainable and circular economy. From now on, greenhouse gas emissions on the scope 3 level must be reported in the EU. In many sectors, all major companies have already recognized this development and identified carbon neutrality as a major goal for the next decade.

At the same time, the "neutralization" of CO_2-emissions by external agencies cannot be more than a bridge until real solutions are established. Not only is the impact of more or less serious providers questionable, but also misleading claims may poison the path for actual improvement.

Despite the force of direct or indirect governmental regulation, consumers expect more and more ecological products and screen critically the ingredients list of cosmetics. The wish for a natural and sustainable lifestyle is a central criterion regarding care and nutrition.

Fermentation, the process of microorganisms converting several carbon sources to various metabolites in a natural process, is well established in producing wine, cheese and bread. As a trend starting in Japan and South Korea, the technique is making its way into the cosmetic industry. While yeast oil is a first-of-its-kind innovation in care products, fermented ingredients are no strangers to consumers. More and more products are entering the market every year. Cosmetics containing sub-

stances processed by microorganisms were forecasted to be a top trend in 2022 [1]. This prediction is backed up by the fact that those products beat up two flies with one flap: sustainability-focused consumers and performance-driven consumers are equally attracted.

Yeasts are heterotrophic organisms, meaning they obtain carbon for growth and energy from complex organic compounds. Their respiration requires oxygen as an oxidizing agent. Consequently, they must be grown in mixed and aerated fermentation vessels. The size of the vessels is between 50 and 250 m^3 in some cases but can also reach 500 m^3. In general, the economy of scale favors larger plants since the cost per m^3 fermenter volume decreases with size. Anyhow, these fermenters are expensive to build in the first place. In addition, the transfer of the yeast culture from the lab to the production vessel must be done in multiple steps to keep the population above a certain threshold. This is typically realized in a series of two or three fermenters with an increasing volume. Furthermore, each fermenter needs to be sterilized beforehand to ensure that only the desired culture is growing. Later, a pH and an aeration control system are used to track and operate the process. The feedstock is usually glucose or a glucose syrup derived from sugar beets, cane or corn.

More commonly known today is another representative of the single cell oil (SCO) producers: algae. In short, algae are microscopic plants that use photosynthesis and, accordingly, the fixation of CO_2 to multiply and grow. Therefore, the key advantage is obviously the direct uptake of CO_2. Additionally, algae are already used to produce polyunsaturated fats, especially Omega-3 fatty acids and antioxidants like β-carotene and astaxanthin. Those components are much more valuable than plant oil alternatives and can therefore be produced economically. The reason for this shortcoming comes with the advantage of taking up CO_2 directly: because of photosynthesis, which requires a constant and well-dosed amount of light, the upscaling process is challenging. To make SCOs economically viable, a production of multiple kilotons of oil per year is needed. Another disadvantage is coupled with this requirement. The growth and space-time-yield of algae are significantly lower compared to yeast. In an optimal scenario, production costs are estimated to be minimal at 5000 €/t [2].

COLIPI's Process

As mentioned before, several resources can be considered to feed yeast cells during fermentation. In a large-scale fermentation, the cost of the substrate has a major impact on the total production cost. To illustrate this problem: let us assume in an optimized process 1/3 of the glucose is converted into TAGs while the rest of the carbon is needed for growth and maintenance of the cell or is burned for energy and therefore blown out as CO_2. If the cost of the glucose would be around 400 €/t, the minimum selling price would already be 1200 €/t, totally neglecting electricity, labor, maintenance and depreciation. Subsequently, the yield must be improved which is possible in certain boundaries by genetically modifying the yeast and/or a cheaper substrate must be found. Many industrial fermentations rely on sugar beet or sugar cane molasses as a cheaper carbon source. Recent research focuses on other agricultural waste streams, promising an almost free and sustainable feedstock. For those materials, including cellulose and hemicellulose, the cost of pretreatment and increased process complexity must be considered. Moreover, a waste stream in constant quality and high quantity throughout the year is rather hard to find.

To overcome these shortcomings, COLIPIs key innovation for the challenging development of a sustainable yet economic process to produce yeast oils is utilizing biogenic or atmospheric CO_2 in a circular process and by this, minimizing the required carbon input from agricultural production or other streams. This concept relies on a second bioprocess, a gas fermentation, which utilizes CO_2 and converts it into biomass as feedstock for the yeast in the main fermentation. The scheme of the process is shown in **Figure 1**. To realize the maximal sustainability, renewable energy will be used. The oil can be used in various applications, from fuels and bulk chemicals via cosmetic and care products to food and feed.

When it is possible to satisfy parts of the oil demand without tropical imports in the first step and decrease the dependency on agrarian production in the second step, the fragile supply chains can be shortened immensely. Moreover, to take more control over a sensitive resource due to a local production can have an invaluable impact in a world possibly moving towards deglobalization and protectionism.

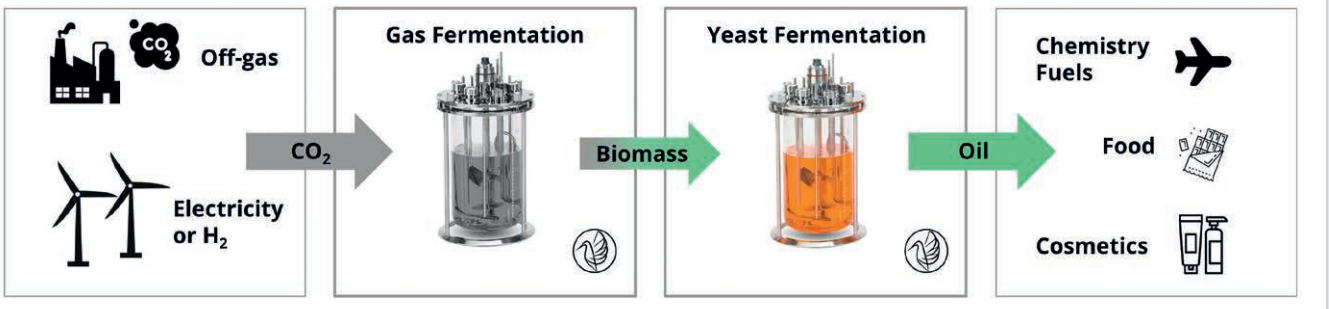

Fig. 1 COLIPIs process for a sustainable oil production.

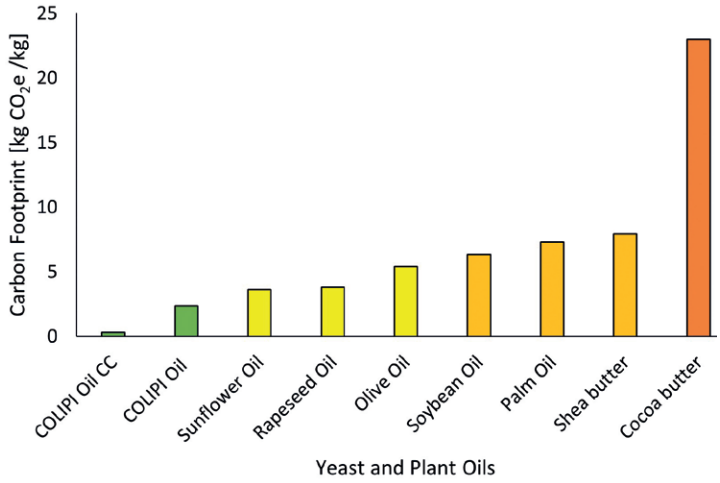

Fig. 2 Carbon footprint of plant oils and butters used in cosmetic products. Values for COLIPI Oil CC (with captured carbon) and COLIPI Oil (without carbon capture but glucose from sugar beets) were calculated by COLIPI and are based on preliminary assumption, for details please contact the authors.

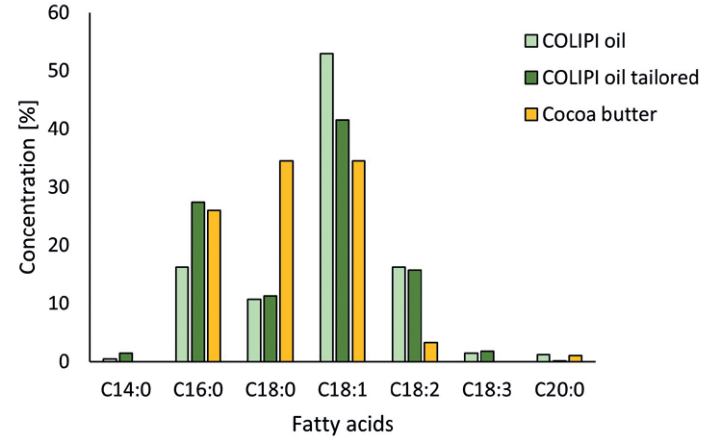

Fig. 3 Fatty acid profile of two COLIPI oil prototypes compared to cocoa butter.

To measure sustainability, several factors can be investigated. Freshwater consumption can be crucial, especially in arid areas. The land use, in general, plays a role since fertile soil is limited and most likely threatened by climate change. These factors should not be excluded from an overall analysis. However, to cover the most prominent dimension and point out some difficulties in the discussion, a closer look at the carbon footprint will be taken in this text. To start, there are two different approaches for life cycle assessments of agricultural products and bioprocesses [3]. The first one takes the CO_2 captured by the plant from the atmosphere during growth into account and considers it stored. Subsequently, the CO_2 taken up is subtracted from the overall process. This is often referred to as "cradle-to-gate" approach. The second one is called the "cradle-to-grave" approach. Here, the CO_2 is released at the end of the product's lifetime in the "grave". Since the balance of the product is net zero, the biogenic CO_2 is excluded from the calculation. Besides those two concepts, there are multiple other approaches with more or less debatable boundaries. The consequence of the lack of a standard method is that different processes should only be compared when the same methods are applied and that transparency is essential. Until today, it is nearly impossible to find reliable data for all materials. In **Figure 2** the carbon footprint of the COLIPI yeast oil and values found in literature for different plant oils are shown. The footprints for the COLIPI oil with and without carbon capture were calculated according to the cradle-to-grave approach. In the case of the COLIPI oil with carbon capture, biomass derived from a gas fermentation based on CO_2 was used as feed. In the case of the COLIPI oil without carbon capture, the glucose was derived from ecologically farmed sugar beets. The oils and butters from sunflower to palm oil are the mean values presented in a review paper [4]. For shea [5] and cocoa butter [6], only very few studies could be found. The collected carbon footprints could be called "cradle-to-fork".

Oil characteristics

In the beginning, the fatty acids most frequently occurring in yeast oil were mentioned. They are the same as those in cocoa butter but in different quantities. **Figure 3** shows the first results of COLIPI's work to tailor the fatty acid profile toward customers' needs. The decreased fraction of oleic acid while increasing the fraction of palmitic acid was achieved without any genetic engineering but with precise fermentation process control.

Among others, "red" yeasts are in the interests of research because of the antioxidative substances they produce. Additionally, the same components are responsible for the orange/red appearance of the microorganisms. Genera like *Rhodotorula* and *Sporobolomyces* contain various oleaginous species being promising to produce highly antioxidative oil. The following **Table 1** shows four values of antioxidative substances found in the literature of wildtype oleaginous yeast *Rhodosporidium toruloides*. In general, carotenoids are divided into the following two classes. Pure hydrocarbons, here represented by β-carotene

Component	Concentration [mg/g_{CDW}]	Reference
β-Carotene	10.0	[T1]*
Astaxanthin	0.1	[T2]*
Torulene	0.3	[T3]*
Torularhodin	0.3	[T4]*

* See References

Table 1: Concentration of antioxidants in wildtype *Rhodosporidium toruloides* according to literature.

and torulene, and xanthophylls, here represented by astaxanthin and torularhodin, which contain oxygen molecules in addition.

Fairly well known are the antioxidant properties of the vitamin A precursor β-carotene. Therefore, it is already heavily used in food and cosmetic industry. Astaxanthin is an even stronger antioxidant and one of the most expensive pigments in feed and cosmetic applications. Torulene and torularhodin have also been shown to have anti-bacterial effects in addition to their antioxidative properties and are associated with tumor apoptosis [7].

Conclusion – Drop-in or effect oil?

As mentioned in the beginning, yeast oil could replace plant oil in many regards. Anyhow, for now and in the near future, most plant oils will have a major advantage: the price. Due to many decades of industrialized farming, plant oils have a considerable lead in optimizing towards low-cost production. Therefore, even though supply chains have been shaken up and the actual agricultural consequences of the Ukraine conflict might show 2023 because of the lack of seeding this year, prices are expected to normalize sooner or later.

Following the economy of scale, yeast oil is quite cost-intensive when produced in small batches. In large scale and maximally-achievable process performance, scientists expect that competitive prizes (appr. 1500 €/t) can be possible once all parameters are optimized [8]. Nevertheless, until processes are established yeast oil will be too expensive to act as drop-in oil. Therefore, because of its outstanding sustainability, the first-of-its-kind marketability and the richness in valuable antioxidants make the oil a fascinating effect oil. To summarize, the roadmap starting from next year is to bring the yeast oil into the market as a differentiator to conventional effect oils to show what could be the green future of cosmetics. In an ongoing process, the production will be scaled up to increase the availability. More products, bigger batches and a growing awareness of customers will follow.

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