



GSH-defense

Boosts endogenous GSH tripeptide to strength skin defensive system

GSH tripeptide in
keratinocytes +50%

Antioxidant capacity
of cell cytosol +50%

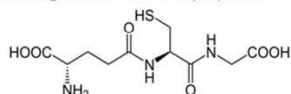
Skin irritation
-20%

Abstract

GSH-DEFENSE is a natural functional ingredient that induces the synthesis of endogenous glutathione tripeptide (GSH), a biomolecule that plays a key role in the defensive system of human cells.

GSH-DEFENSE is produced with a patented technology based on the rational use of enzymatic biocatalyzers that conjugates cutting edge technologies with the leading principles of Green Chemistry and environmental sustainability. Thanks to these technologies it is possible to recover the complete phytocomplex of plants in a highly bioavailable form using just water as the solvent.

endogenous GSH tripeptide



GSH-DEFENSE contains deglycosilated isothiocyanates from rocket salad, able to induce the production in skin cells of endogenous glutathione tripeptide, effective in strengthening the natural defensive barrier against radicals and toxic compounds due to its protective effects. This natural tripeptide can intervene in a broad spectrum of biological reactions that detoxify, protect and repair cells, keeping skin strong and more able to efficiently react to impairments. The high levels of bioavailable isothiocyanates, released in water, are able to increase the content of glutathione in keratinocytes up to 50%, drastically improving the antioxidant capacity of cell cytosol.

GSH-DEFENSE naturally boosts the inner defenses of the skin, and *in vivo* tests have shown that is able to reduce an induced irritation acting as an effective lenitive product.

Manufacturers can use this extremely easy to use, cost effective and safe (food grade) product, able to stimulate the synthesis of the most important natural tripeptide of the skin, to develop effective cosmetics, following the peptide trend but without the use of any synthetic product.


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Introduction

Brassicaceae are an important family of flowering plants, also known as the crucifers or the cabbage family. These plants are characterized by the presence of a group of secondary compounds called glucosinolates. Many glucosinolate degradation products, called isothiocyanates, are of interest because of their biological activities due to the chemical group $-N=C=S$.

Isothiocyanates are potent inducers of phase 2 detoxification enzymes and it is now widely recognized that the up-regulation (induction) of antioxidant molecules, such as glutathione (GSH), is a powerful and highly efficient strategy for protection against the damaging effects of reactive oxygen intermediates and electrophiles.

Recent studies investigated the effect of a topical application of isothiocyanates. The product applied on skin produced a very interesting dose-dependent increase of the phase 2 defensive systems of the skin. The up-regulation of the phase 2 enzymes is associated with a higher self-protecting ability of the skin from cellular damage caused by reactive oxygen species such as free radicals, peroxides and other oxidative stress, UV radiation, etc. These features of isothiocyanates were also tested topically by applying a plant extract and measuring the reduced susceptibility of the skin to UV induced erythema.

To develop a new active ingredient for cosmetic purposes containing isothiocyanates it was necessary to select the starting plant in order to obtain the highest possible amount of active molecules, and thus the best possible efficacy of the final product. Rocket salad (*Eruca sativa*) showed the highest content in isothiocyanates, with a concentration twice that observed in broccoli, has no unpleasant smell and is produced throughout the year as a fresh and standardized product. This makes it possible to guarantee a reliable supply chain of a high quality starting material that can be processed fresh in order to preserve the characteristics of the plant at its best.

In plants, the releasing system of isothiocyanates is mediated by an enzyme class called myrosinases. To release isothiocyanates is mandatory to perform a hydrolysis process with enzymes that have a myrosinase-like activity. Phenbiox's Bioliquefaction Technology is able to completely release the isothiocyanates from the glucosinolates contained in the plant. The bioliquefied rocket salad therefore contains 100% of bioactive isothiocyanates.

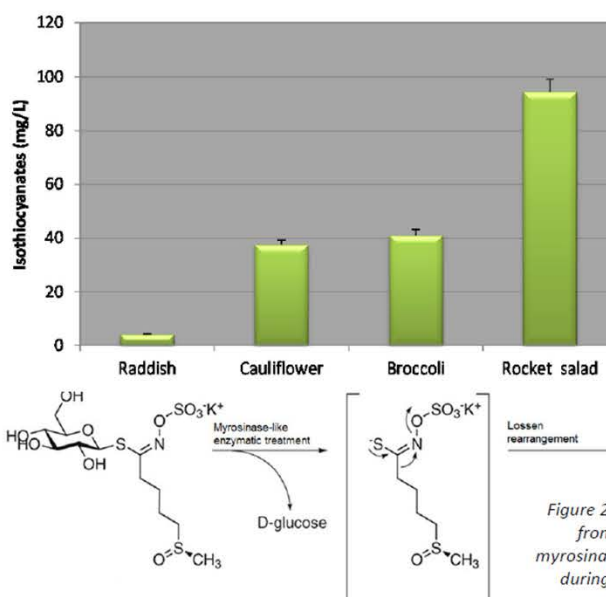


Figure 1: total content of isothiocyanates in Brassicaceae.

Figure 2: Release of isothiocyanates from glucosinolates catalyzed by myrosinase-like enzymatic treatment during the bioliquefaction process

Thanks to the enzymatic treatment of Phenbiox's patented Bioliquefaction Technology, using rocket salad it was possible to develop **GSH-DEFENSE**, a new active ingredient for cosmetics containing isothiocyanates in their biologically active form.

GSH-DEFENSE: boosting glutathione synthesis

GSH is the most important and abundant endogenous tripeptide antioxidant in humans.

Vitamin C, vitamin E, phenols and carotenoids are exogenous direct antioxidants. These molecules have to be taken with the diet and are able to neutralize free radicals by chemically quenching the electrons. Instead, glutathione is self-synthesized by cells and promotes the detoxification of several dangerous species, both quenching electrons and through enzymatic systems.

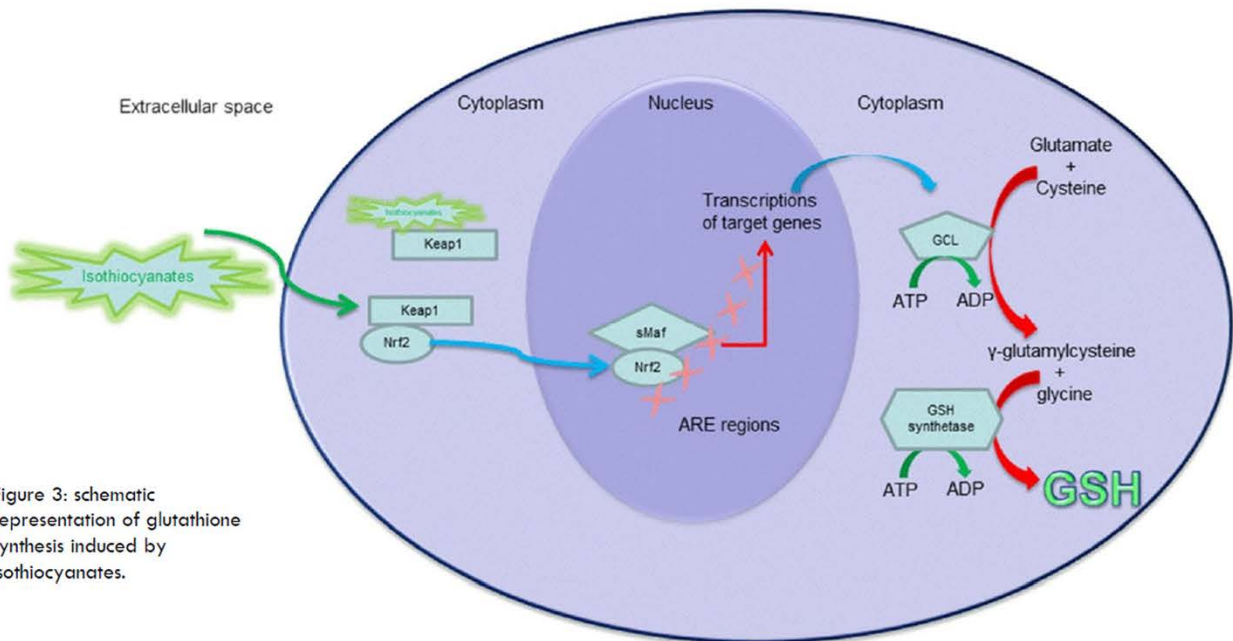
Glutathione participates directly in the neutralization of free radicals and reactive oxygen compounds, as well as maintaining exogenous antioxidants such as vitamins C and E in their reduced (active) forms. It is used in metabolic and biochemical reactions such as DNA synthesis and repair, protein synthesis, prostaglandin synthesis, amino acid transport and enzyme activation. Thus, GSH plays a prominent role in the detoxification and/or excretion of hazardous molecules and in contrasting the inflammatory processes often related to radical presence.

Proper delivery of isothiocyanates to cells is an easy and effective way to strengthen the natural defensive barrier against harmful radical species and toxic compounds due to the protective effects of glutathione.

GSH-DEFENSE is able to boost the response regulation of glutathione synthesis through an over expression of glutamate-cysteine ligase (GCL), a rate limiting enzyme that catalyzes the GSH synthesis.

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GSH-DEFENSE: *in vitro* testing

The effect that the isothiocyanates of GSH-DEFENSE has in terms of promoting the synthesis of glutathione in keratinocytes was measured. In just 24 hours the keratinocytes treated with GSH-DEFENSE are able to drastically increase, in a dose dependent way, the total content of glutathione up to 50% with respect to the basal content of untreated keratinocytes. This higher concentration of glutathione leads to a higher capacity of the cell to counteract oxidative stress and other hazardous conditions, and therefore to better recover from irritations.

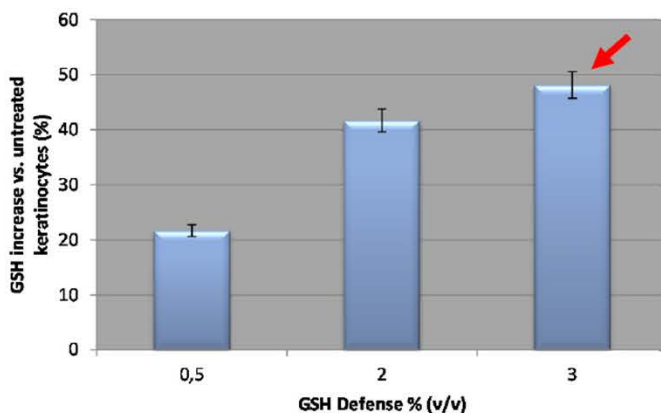


Figure 4: increase of glutathione content in keratinocytes after a 24 h treatment with different percentage of GSH Defense.

GSH tripeptide in keratinocytes +50%

An *in vitro* evaluation of the antioxidant capacity of the cytosol of keratinocytes treated with GSH-DEFENSE was also performed. When keratinocytes are treated for 24 hours with 3% v/v of GSH-DEFENSE, a drastic increase of antioxidant capacity, close to 50% more than the untreated keratinocytes, is observed. The treatment of keratinocytes with 3% (v/v) of GSH-DEFENSE is ideal to enhance the production of natural protective peptides such as glutathione, improving the capacity of skin cells to be self-protected from external hazardous conditions.

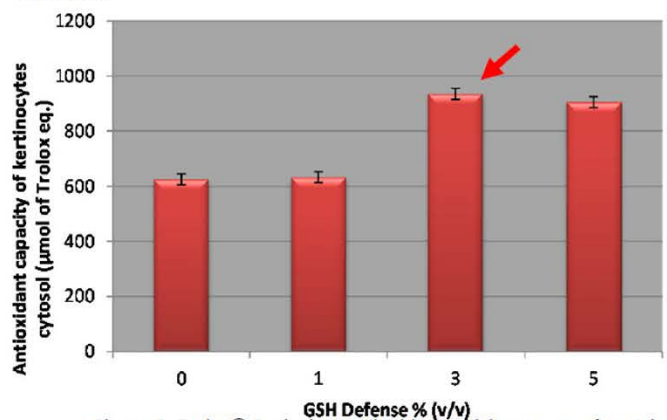


Figure 5: Trolox® Equivalent Antioxidant Activity per mg of protein (TEAA μmol/mg protein) of keratinocytes treated with different concentrations of GSH-DEFENSE for 24 h.

Antioxidant capacity of cell cytosol +50%

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GSH DEFENSE: *in vivo* testing

In order to verify whether the results observed with the *in vitro* testing are correlated to measurable effects on skin, a set of *in vivo* tests was outlined.

Tests were performed to evaluate the lenitive effect of GSH-DEFENSE on a mild irritation caused by sodium lauryl sulfate (SLS). The study was performed on thirteen healthy volunteers of both sexes aged from 25 to 65. An occlusive patch with SLS solution was applied onto the volar surface of the forearm of each volunteer. The patch was removed after 24 hours and on testing points were used a simple cosmetic containing the 3% (w/w) of GSH-DEFENSE and a placebo formulation containing water instead of the GSH-DEFENSE. The product was applied twice per day and the redness reduction was measured.

The areas treated with the cosmetic formulation containing GSH-DEFENSE showed a much faster reduction of the irritation caused by the occlusive application of SLS. In 48 hours, after 4 applications of the GSH-DEFENSE, the reduction was drastic compared to the placebo formulation, -20 %. The data were also highly significant, as the values obtained with a Student t test were $p < 0.01$.

The data obtained show that GSH-DEFENSE is very effective in improving the capacity of skin to recover after stress, as the product is able to quickly and effectively enhance the defensive response of keratinocytes.

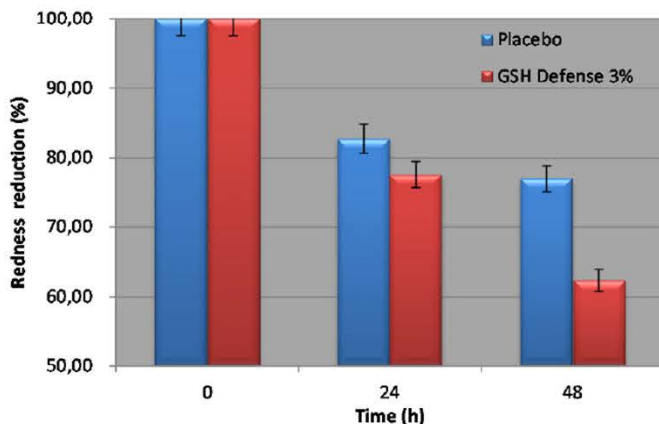


Figure 6: redness reduction of SLS induced irritation on skin treated with GSH-DEFENSE 3% vs. a placebo formulation.

Skin irritation -20%

GSH-DEFENSE: cosmetic applications

GSH-DEFENSE is an easy to use active ingredient for cosmetic applications.

GSH-DEFENSE is an extremely safe product that can be used for all skin types, even the most sensitive.

GSH-DEFENSE can be part of any cosmetic formulation containing water and can be added under both hot and cold conditions during cosmetic preparation.

GSH-DEFENSE can be used in skin care and hair care:

lenitive/anti redness products;

pre sun, sun and after sun products;

detoxifying products;

preventive anti-age products;

Suggested for:

Skin Care (face and body cream and lotion, shaving and after shave products, gels, professional treatments, etc.);

Hair Care (hair lotion, scalp treatments, hair creams and masks, etc.).

GSH-DEFENSE technical specifications:

CTFA name: WATER (and) HYDROLYZED ERUCA SATIVA LEAF

Ingredients: water, Eruca sativa (leaves), citric acid, sodium benzoate, potassium sorbate.

Suggested concentration of use: 3% w/w

Solubility: soluble in water, alcohol, glycerin

pH: 2.5 – 3.5

Stable in formulation with pH from 1 to 8



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