# SulforaWhite: Garden Cress Sprout Fraction with Strong Whitening Activity

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## Abstract

An extract of garden cress sprouts that was incorporated into a liposomal skin carrier system, was found to exhibit a strong whitening effect. This new whitening ingredient, SulforaWhite, is characterised by a standardised content of sulforaphane, the well-known phytonutrient of Brassicaceae vegetables. In vitro, the whitening efficacy was demonstrated on B16 melanocytes after stimulation with α-MSH. The result could be clearly reproduced in vivo with a cream containing 2% SulforaWhite, tested on 21 Asian subjects for 56 days.

## Introduction

In Asia there is a broad demand for whitening products, either to lighten the skin complexion generally or to adjust differences in pigmentation. For Caucasian skin, whitening products are used to treat age spots or other forms of hyperpigmentation like freckles or darkly pigmented scars. What nowadays every woman wishes is a porcelain complexion. There is a trend to brighten up the skin to give an even, radiant tone. Skin lightening is seen as part of the anti-aging skin care.

The process from exposure to UV light to pigmentation is very complex and contains many steps. As shown in Figure 1, UV light leads to the generation of reactive oxidants in keratinocytes. This causes the keratinocytes to release inflammatory mediators such as prostaglandins and NO and the alpha-melanocyte stimulating hormone (α-MSH). There are receptors for prostaglandins and α-MSH on melanocytes. A lot of research was done on the receptor for α-MSH, called melanocortin 1 receptor (MC1R). After binding with α-MSH, the receptor induces melanocytes to promote the expression of the tyrosinase gene and to enhance dendricity. Tyrosinase is the rate-limiting enzyme in the synthesis of melanin pigments. Melanin is produced in specialised organelles, called melanosomes. These organelles are gradually filled with pigments, transported to the peripheral dendrite tips and transferred to the surrounding keratinocytes. There, melanosomes form a protective shield around the cell nucleus, producing a uniform skin colour.

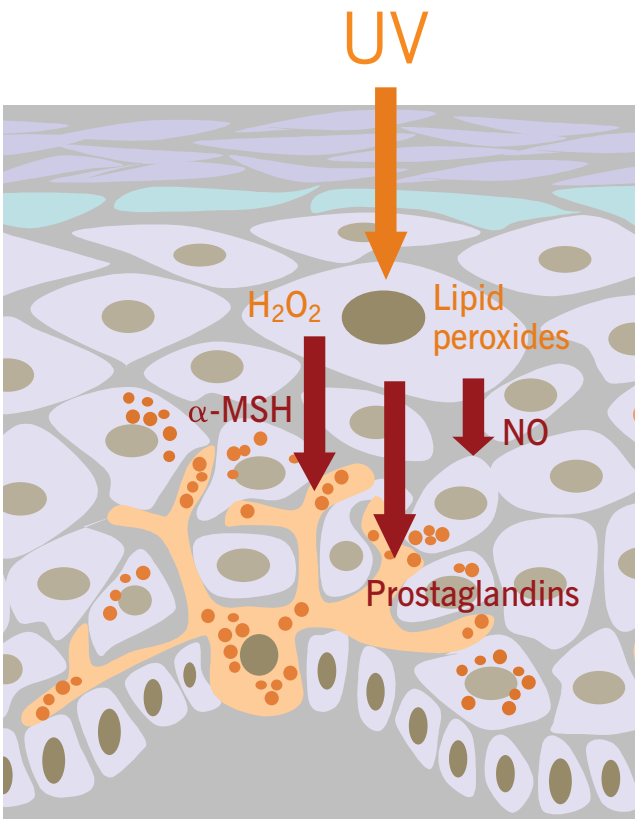


Figure 1 Signalling mediators involved in the tanning response

In the past, pigmentation was inhibited mainly by actives that reduced the enzymatic activity of tyrosinase. The whitening actives marketed today interfere at different steps in the pigmentation cascade. A series of new actives came up that were reported to block the transfer of melanosomes to keratinocytes. Another efficient way to suppress pigmentation would be to block the upregulation of the expression of tyrosinase and to block the stimulation of melanocyte dendricity. This could be achieved by interfering with the binding of keratinocyte mediators to their receptors on melanocytes or by inhibiting the production of these mediators.

The sulforaphane phytonutrients in SulforaWhite are known for their capacity to neutralise reactive oxidants. Sulforaphane promotes the enzymatic defence system against oxidants. The whitening mechanism of SulforaWhite, or at least part of it, is therefore to block the formation of reactive oxidants by UV and hence the resulting induction of pigmentation mediators.

## Garden cress sprouts: a rich source of sulforaphane

Garden cress has a spicy aroma and a refreshing, peppery-pungent taste. Like the other members of the Brassicaceae family, garden cress owes its aroma to isothiocyanates. They form a class of phytonutrients that are linked to the anti-cancer health benefits of Brassicaceae vegetables. Well known members include cabbage, broccoli, cauliflower, kale, rape-seed, mustard, radishes, horse radish, water and garden cress. The sprouts of these vegetables contain the highest concentration of phytonutrients. Sprouts are nowadays very popular as food supplements. The publicity on sprouts was especially driven by the research on the isothiocyanates of broccoli sprouts done at the Johns Hopkins University. The concentration of the anti-cancer isothiocyanate active was found to be 20 to 50 times higher in 3-day-old sprouts than in mature broccoli[1]. Isothiocyanates are sulphur-containing compounds that are present in the living plant as glucose-derivatives, called glucosinolates. The molecular structures are shown in Figure 2. When the vegetables are chewed, the plant cells are broken and the enzyme myrosinase is liberated that hydrolyses the glucosinolates into isothiocyanates. The predominant isothiocyanate in garden cress as well as in broccoli is sulforaphane.

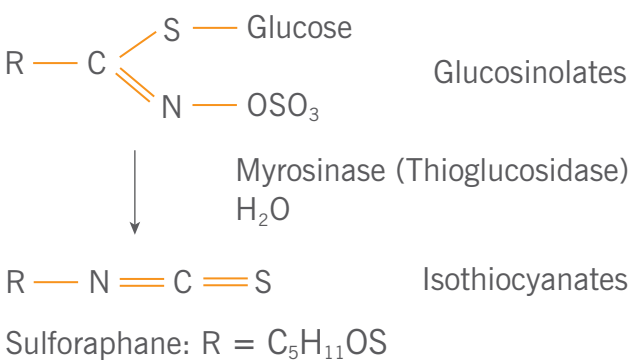


Figure 2 Formation of isothiocyanates and chemical formula of sulforaphane

## The capacity of sulforaphane to neutralise reactive oxidants

In past years, a lot of research was done on the mechanism of the anti-cancer activity of sulforaphane. It was shown to act as an indirect antioxidant by enhancing the activity of phase II enzymes and antioxidant enzymes. This is an enzymatic defence system against reactive, toxic substances such as electrophiles and oxidants. The research group that contributed the most to the anti-cancer mechanism of broccoli sprouts also published very recently a paper about the protective effects of sulforaphane against damage by UV radiation[2]. They showed that topically applied sulforaphane reduced susceptibility to erythema by mobilisation of the cellular enzymatic defence system.

The capacity of SulforaWhite to promote the expression of antioxidant enzymes was analysed in vitro using normal human epidermal keratinocytes. The method of real-time polymerase chain reaction (PCR) was used to measure the expression of selected genes. Several antioxidant enzymes were chosen as representatives of phase II enzymes. NADPH:quinone reductase 1 (NQO1) is a major anti-carcinogenic enzyme with a principal role in transforming quinones into stable hydroquinones. Heme oxygenase 1 (HO-1) is induced after exposure to oxidative stress, such as UV irradiation or hyperoxia, indicating its role in cellular defence. Thioredoxin reductase 1 (TrxR1) works together with NADPH to control the redox balance of the cell. Glutathione peroxidase (GPX1) enzyme has a major role in the reduction of lipid peroxides and of free hydrogen peroxide. The keratinocytes were grown in standard growth medium to 80% confluence. Then the cells were incubated for 24 hours with 0.05 or 0.2% SulforaWhite. After incubation, the cells were harvested and total RNA was extracted. Compared to the untreated control, the antioxidant enzyme NQO1 was moderately stimulated at 0.05% and strongly stimulated at 0.2% SulforaWhite (Table 1). HO-1 and TrxR1 were both stimulated strongly even at the lower SulforaWhite concentration. The enzyme GPX1 did not respond to SulforaWhite in this trial.

| Concentration of SulforaWhite (%) | 0.05 | 0.2 |
|---|---|---|
| | Expression (%) to untreated | |
| **Phase II enzymes** | | |
| NAPDH:quinone reductase 1 | 175 | 314 |
| Heme oxygenase 1 | 312 | 4282 |
| Thioredoxin reductase 1 | 284 | 2416 |

Table 1 Effect of SulforaWhite on expression of antioxidant enzymes

## SulforaWhite: A whitening ingredient based on cress sprouts

Garden cress is suitable for hydroponic cultivation and is typically harvested just a week after germination. 4 to 5 day old garden cress sprouts were used as the raw material to produce SulforaWhite. The composition (INCI) is: Lepidium Sativum Sprout Extract, Glycerin, Lecithin, Phenoxyethanol and Aqua. For a better skin uptake, the actives of SulforaWhite are incorporated into liposomes. In the sprouts, sulforaphane is present as glycoside, called glucoraphanin. For analysis of the sulforaphane content, the sprout extract was first treated with myrosinase to hydrolyse the glycosides and the resulting sulforaphane was measured by HPLC after cyclocondensation with 1,2-benzenedithio[3]. The concentration of sulforaphane in SulforaWhite was standardised to about 100 $\mu$M.

## Study results on the whitening efficacy of SulforaWhite

Inhibition of melanin formation was confirmed in a cell-based assay using B16 murine melanoma cells. Cultivation was done in 96 well-plates for 72 hours in the presence of a stable derivative of $\alpha$-MSH. After incubation, the melanin content was analysed by measuring optical density at 405 nm. A plate that was cultivated in parallel was used for the evaluation of cell viability by the MTT assay. SulforaWhite was tested at three different concentrations. Melanin formation was strongly inhibited at 0.4%. The results are illustrated in Figures 3 and 4. The MTT assay clearly demonstrated that this was not the consequence of a cytotoxic effect. The inhibitory effect of SulforaWhite on melanin formation after stimulation with $\alpha$-MSH could also be demonstrated with normal human melanocytes. SulforaWhite at 0.016% reduced melanin synthesis by 47%.

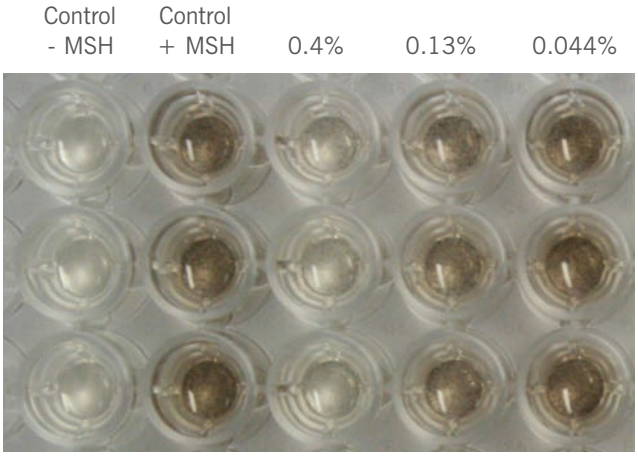


Figure 3 Incubation plate with the B16 murine melanocytes in triplicates

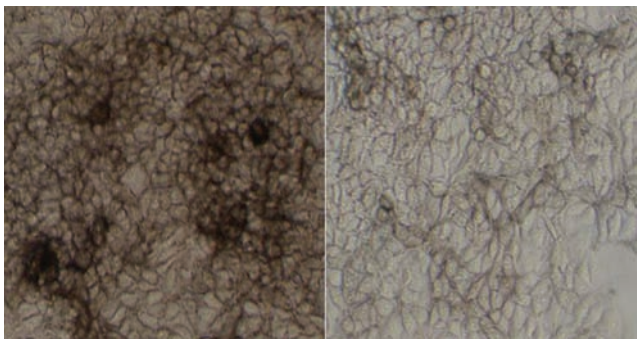

Figure 4 Microscopic photos of the B16 murine melanocyte monolayers (left: control with -MSH; right: with 0.4% SulforaWhite)

A human clinical trial was conducted on 22 Asian subjects aged between 22 and 39. A cream with 2% SulforaWhite was applied twice daily for 56 days on the inner side of one forearm. The other forearm was treated with the placebo cream. The upper arm was used as an untreated zone. Skin colour was measured with the chromameter MINOLTA type CR300. Whitening is shown by increased skin clarity, measured as lightness L*, and by an increased Individual Typological Angle (ITA) degree. For illustration of the visual effects macrophotographs were made. The chromameter results showed a clear placebo controlled whitening effect. Refer to Figure 5. After 56 days of use, and compared to the

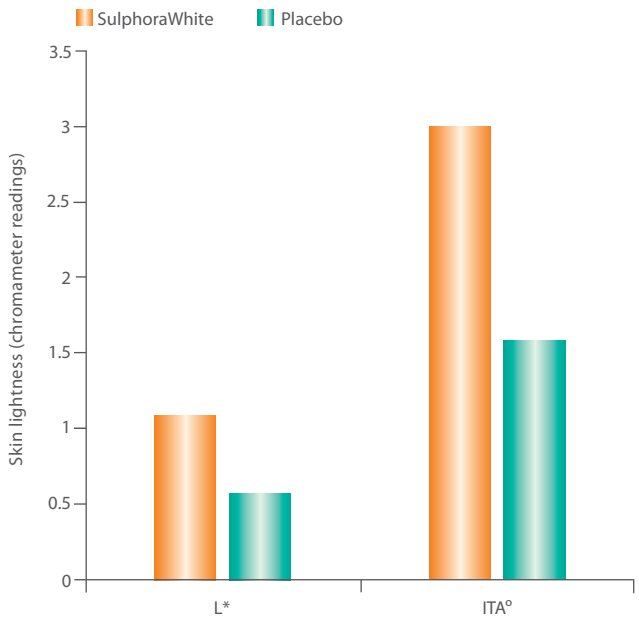


Figure 5 Lightness parameter L* and ITA° value after 56 days

placebo product, the cream with SulforaWhite induced a significant increase in lightness L* (+ 0.5 ± 0.2 A.U.; p = 0.004) and a significant increase in the ITA° parameter (+ 1.4 ± 0.4 A.U.; p = 0.004). Figure 6 (below) demonstrates that the whitening effect was visible to the naked eye.


Figure 6 Photos of the inner side of the forearms of one subject

## Conclusion

A garden cress sprout extract was demonstrated to exert a significant whitening activity. A couple of in vitro studies were performed in order to analyse the whitening mechanism. SulforaWhite was not active in assays with isolated human tyrosinase. The results of the cell-based assays with B16 cells or the normal human melanocytes therefore indicate that SulforaWhite antagonises binding of a-MSH to its receptor on melanocytes. A recent scientific publication showed that the isothiocyanate sulforaphane reduced the susceptibility to UV radiation-induced erythema. The whitening mechanism of SulforaWhite seems to be linked also to its capacity to protect against reactive oxidants, the very first triggers in the pigmentation cascade. This way, SulforaWhite exerts a skin whitening effect and protects at the same time against premature skin aging.

## References

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## Author Biography

Dr. Daniel Schmid received his Ph.D. in biochemistry from the University of Zurich, Switzerland. He worked for several years in the Research Centre of Nestlé at Vers-chez-les-Blanc, where he studied health effects of probiotic bacteria. He is presently responsible for research in Mibelle Biochemistry, a business unit of Mibelle AG Cosmetics. Mibelle Biochemistry develops and produces active ingredients for skin care products.