Abstract
Cress sprouts were used as the source material for the development of a cosmetic ingredient rich in isothiocyanates. The scientifically well established efficacy of isothiocyanate phytonutrients to detoxify environmental pollutants and to neutralize reactive oxygen species, could be reproduced in an in vitro skin model. The ingredient was found to stimulate the cells’ inherent detoxification system and skin cells pre-treated with the cress sprout ingredient proved to be more resistant to toxic oxidants. In a study during the winter period with twenty subjects that worked mainly outdoor where they are exposed to low humidity, cold winds and pollution, the cress sprout ingredient was found to increase skin firmness and smoothness compared to placebo.

Introduction
Mom was right: “Eat your vegetables, they are good for you!” Vegetables have been linked to many health benefits, including lowered risk for certain cancers, stroke, heart disease, and high blood pressure. Vegetables in the mustard (Brassicaceae) family have extra health value. They have been proved in numerous studies to help lower the risk of colon, stomach, lung and prostate cancer. Isothiocyanates, a class of plant chemicals that are characteristic of the Brassicaceae family, have been identified as the anticancer actives.

Sprouts have naturally occurring levels of phytonutrients higher than any whole food. 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 (Fig. 1). When the vegetables are chewed, the plant cells are broken and the enzyme myrosinase is liberated that hydrolyses the glucosinolates into isothiocyanates.

In the past years, a lot of research was done on the mechanism of the anti-cancer activity of the isothiocyanate sulforaphane. It was shown to act as an indirect antioxidant by reducing the activity of phase I enzymes and by enhancing the activity of phase II enzymes (2). These enzymes are part of two main types of metabolism that deal with the elimination of xenobiotics (e.g. environmental pollutants), drugs and carcinogens (Fig. 2).
In general, phase I enzymes catalyze reactions that increase the reactivity of hydrophobic compounds, preparing them for reactions catalyzed by phase II enzymes. The latter generally increase water solubility and promote the elimination of the compound from the body. Nowadays, antioxidant enzymes are also regarded as part of the phase II metabolism.

The genes of classical phase II enzymes and antioxidant enzymes contain a specific sequence of DNA called an antioxidant response element (ARE). Isothiocyanates have been found to increase the transcription of genes containing a promoter with an ARE sequence. The exact mechanism is shown in Figure 3. The transcription factor Nrf2 is the critical regulator of ARE-dependent transcription. Under basal conditions, Nrf2 is largely bound in the cytoplasm to Keap1. As a heterodimer, Nrf2 is inactive. But disruption of the Nrf2-Keap1 complex leads to nuclear translocation of Nrf2, binding to the ARE sequence and expression of detoxification and antioxidant enzymes. The isothiocyanates are speculated to activate this pathway either by specific interaction with Keap1 and thereby liberating Nrf2 (3) or by induction of Nrf2 expression and suppression of that of Keap1 (4).

A Cosmetic Ingredient Based on Garden Cress Sprouts

Garden cress displays a spicy aroma and a refreshing, peppery-pungent taste. Like the other members of the Brassicaceae family, garden cress owes its aroma to isothiocyanates. Garden cress is suitable for hydroponic cultivation and typically harvested just a week after germination. 4 to 5 day old garden cress sprouts were used as the raw material to produce the cosmetic ingredient Detoxophane. The composition of the ingredient is the following (INCI): Lepidium Sativum Sprout Extract, Glycerin, Lecithin, Phenoxyethanol and Aqua.

For a better skin uptake, the actives of Detoxophane 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 hydrolyze the glycosides and the resulting sulforaphane was measured by HPLC after cyclocondensation with 1,2-benzenedithiol (5). The percentage of sulforaphane in the ingredient was standardized at 0.005 to 0.012%.

Protection against Oxidative Stressors

Detoxophane was found to positively modulate the expression of phase I and phase II enzymes (results not shown). As consequence, cells in culture pretreated with Detoxophane should be more resistant against toxic chemicals. This protective effect of Detoxophane was analyzed in vitro using normal human epidermal keratinocytes. The chemical stressor used was tert-butyl hydroperoxide (t-BH) which is a strong oxidizing organic peroxide.

The keratinocytes were precultured in standard growth medium. Then the cells were pre-treated for 24 hours with 0.05 % Detoxophane. After this pre-treatment, the culture supernatant was removed and standard growth medium with t-BH was added. After 4 hours’ incubation, the cell viability was measured by MTT assay. Cell viability was analyzed again 20 hours after incubation with the stressor. Detoxophane exerted an impressive protection against t-BH (Fig. 4).

The IC$_{50}$ value, indicating the concentration of the stressor inducing 50 % mortality, increased from 258 µM in the control without Detoxophane to 646 µM in the culture pre-treated with Detoxophane. The protective effect persisted even after 20 hours after incubation with t-BH with an IC$_{50}$ value of the control of 146 µM shifted to 236 µM with Detoxophane.
Protection against winter skin problems

A cream with 2% Detoxophane was tested in a study over eight weeks during the winter period on twenty subjects that worked mainly outdoor. The test product was applied twice daily on the inner side of the forearm. The placebo cream was applied on the other arm. Skin firmness was measured with the Cutometer SEM 475 (Courage & Khazaka GmbH, Cologne) and skin roughness with the PRIMOS device (GFMesstechnik GmbH, Teltow, Germany). Compared to the placebo treated skin area, the skin treated with the Detoxophane cream was after eight weeks clearly firmer and smoother (Fig. 5 and 6).

Conclusion

Environmental stressors are the cause of premature skin aging. Beside ultraviolet radiation, the most prominent stressors are xenobiotic pollutants but also drugs and certain stimulants. Especially during winter, environmental aggressions such as low humidity, icy temperatures and wind combined with high air pollution in the citys are a big threat for the skin. Isothiocyanate phytonutrients help to eliminate these stressors. By inhibition of phase I enzymes they reduce the oxidative transformation of precursor compounds into toxic intermediates. As inducer of phase II enzymes they promote the elimination of toxic intermediates by excretion and the enzymatic neutralization of oxidants. Isothiocyanates are therefore much more than just single antioxidants. They offer a broad protection against chemical aggression. Topically applied isothiocyanates will protect skin cells against DNA damage and thus prevent apoptosis (cell death), the typical mechanisms leading to premature skin aging.

References


(3) Zhang D.D. & Hannink M. (2003) Distinct Cysteine Residues in Keap1 Are Required for Keap1-Dependent Ubiquitination of Nrf2 and for Stabilization of Nrf2 by Chemopreventive Agents and Oxidative Stress. Mol Cell Biol 23(22):8137-51
