



Daniel Schmid

Vegetable sprouts: a potent source for cosmetic actives

DANIEL SCHMID*, RIIKKA SACHER, ESTHER BELSER, FRED ZÜLLI

*Corresponding author

Mibelle Biochemistry, Bolimattstrasse 1, Buchs, 5033, Switzerland

ABSTRACT: Vegetable sprouts are a very rich source for vitamins and physiologically active secondary metabolites. Cosmetic ingredients based on sprouts of different vegetables have been found to exert specific benefits in the skin. An active prepared from garden cress sprouts was shown to work as a general anti-aging ingredient by stimulating the cell's own defense system against free radicals. An extract of mustard sprouts, known to stimulate blood circulation, was found to increase the lip volume after topical application, and a preparation of sunflower sprouts turned out to be efficient in enhancing the cellular energy in the skin.

SPROUTS ARE A RICH SOURCE OF HEALTH PROMOTING PHYTOCHEMICALS

Cosmetic ingredients based on plant raw materials have become a major trend in recent years. There is also a strong demand for functional ingredients with benefits beyond traditional moisturizing. Ingredients should exert physiologic effects leading to improved skin firmness and wrinkle reduction. Other functional ingredients are developed to treat uneven pigmentation or for overall skin whitening. The plant kingdom is a reservoir of biologically active chemicals. Such compounds could work as the active component in a functional cosmetic ingredient. Very interesting in this respect are the so called "secondary metabolites". These are plant compounds that are not required for the primary metabolic processes like growth and reproduction. The role of secondary metabolites in plants is to protect them from disease, damage, pathogens, drought, salinity, extreme ultraviolet and pollutants. Secondary metabolites include substance families like flavonoids, saponins, monoterpenes, phytosterols and isothiocyanates. Many of these phytochemicals are known to exert beneficial effects on human health or to play an active role in the amelioration of disease. Well known examples are the healthy phytochemicals of red wine grapes, the antioxidant procyanidins that help against cardiovascular diseases (1). Vegetable sprouts are the young shoots that develop from germinating seeds. The shoots are known as the plant material with the highest level of healthy

nutrients. Plant seeds contain the embryo and stored food reserves. When under favourable conditions the seeds begin to germinate, the food reserves are mobilized. The fats are transformed into free fatty acids, starch into maltose and proteins into free amino acids. At this stage, some other very important nutrients start coming up in the growing seed, such as vitamins, enzymes and secondary metabolites. Sprouts that are a few days old have the highest concentration of healthy nutrients per calorie of any food (2). Because the shoots of a plant are especially vulnerable, secondary metabolites are very abundant at this stage. The sprouts of a certain species or plant family produce specific chemical types of secondary metabolites. The goal of the work described in this article was to find sprouts of selected species with specific benefits when used as a basis for cosmetic ingredients. Sprouts or shoots can be easily produced by hydroponic cultivation. The seeds are first soaked in water and left 2 days to start sprouting. The very small shoots are then transferred to rotating containers that provide drainage and aeration. Rotation is important to prevent the development of micro climates that favour bacterial and fungal contamination. After a couple of days' incubation in the containers, the shoots are harvested (Figure 1). Shoots are produced in the dark in order to inhibit the production of chlorophyll pigments.

GARDEN CRESS SPROUTS FOR REDOX AND RADICAL CONTROL

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 (3). Isothiocyanates are sulfur-containing secondary metabolites that are present in the living plant as glucose-derivatives, called glucosinolates (Figure 2). When the vegetables are chewed, the plant cells are broken and the enzyme myrosinase is liberated that hydrolyses the glucosinolates into isothiocyanates. Glucosinolates are typical for vegetables of the Brassicaceae family that include well known members such as cabbage, broccoli, cauliflower, kale, rape-seed, mustard, radishes, horse radish, water and garden cress. Sulforaphane is the best characterized isothiocyanate in broccoli (3). It gives them a spicy aroma and a refreshing, peppery-pungent taste. Sulforaphane is a well described, natural activator of the transcription



Figure 1. Shoots of sunflowers that germinated 10 days in a hydroponic cultivation system.

factor NF-E2-related factor 2 (Nrf2), that plays an essential role in the cellular defense against highly reactive chemicals (4). Free radicals generated by oxidative stress and electrophilic, toxic compounds represent the main threat at the cellular level. Our cells respond to these toxic chemicals by increasing the synthesis of cytoprotective proteins. These are detoxifying proteins and enzymes that rebalance the redox status by neutralizing electrophiles and replenishing used cellular antioxidants such as glutathione. These cytoprotective proteins are characterized by a specific gene sequence, called antioxidant response element (ARE). The expression of these proteins is regulated by the transcription factor Nrf2 that binds to the ARE site in the promoter regulatory sequence (5). Recent scientific publications also demonstrate for Nrf2 a key role in the UV response of the skin. UVA, that is known to generate a significant oxidative stress, was found to induce the expression of the protecting, ARE-containing enzyme heme oxygenase 1 (HO-1) (6).

Mibelle Biochemistry used garden cress sprouts as a raw material to produce a cosmetic ingredient with the active sulforaphane (Detoxophane). For a better skin uptake, the actives of the cress sprout ingredient were incorporated into liposomes. The capacity of Detoxophane to promote the expression of cytoprotective proteins was studied in normal human keratinocytes using quantitative PCR for expression analysis (Figure 3). 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 defense. Thioredoxin reductase 1 (TrxR1) works together with NADPH to control the redox balance of the cell. Compared to the untreated control, the antioxidant enzyme NQO1 was moderately stimulated by the cress sprout ingredient (114 percent increased). HO-1 and TrxR1 were both stimulated strongly, by 42 fold and 23 fold respectively. Glutathione peroxidase (GPX1) that has a major role in the reduction of lipid peroxides and of free hydrogen peroxide, did not respond to the cress sprout ingredient in this study.

MUSTARD SPROUTS TO INCREASE LIP VOLUME

Yellow mustard (*Sinapis alba*), another member of the Brassicaceae family, is a herbaceous annual plant that grows to about 60 cm

in height. Its yellow flowers develop into cylindrical hairy pods filled with yellowish seeds used as raw material for mustard production. The glucosinolate sinalbin is the typical secondary plant metabolite of yellow mustard. The myrosinase product of sinalbin, 4-hydroxybenzyl isothiocyanate is responsible for the typical pungent taste of mustard (Figure 4). When applied to the skin or mucous membranes, the mustard isothiocyanate causes a dilation of the capillaries and an increase in blood circulation inducing redness and a feeling of warmth (7). An aqueous extract of 7 days old yellow mustard sprouts (LlPerfection) as a natural, safe blood flow enhancer was tested for stimulation of lip volume. A cream with 2 percent of the mustard extract was applied on the lips twice daily over two weeks by 18 women. The same group of people applied also only the placebo cream during two other weeks. Compared to placebo, the mustard ingredient was found to clearly increase the lip volume by 50.1 mm³ (Figure 5). The effect could be nicely demonstrated by digital photos (Figure 6). Compared to placebo, the mustard ingredient was also shown to induce an immediate hydration increase of 10 percent (not shown).

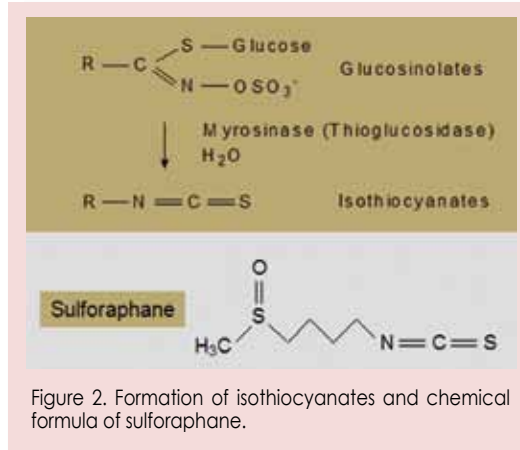


Figure 2. Formation of isothiocyanates and chemical formula of sulforaphane.

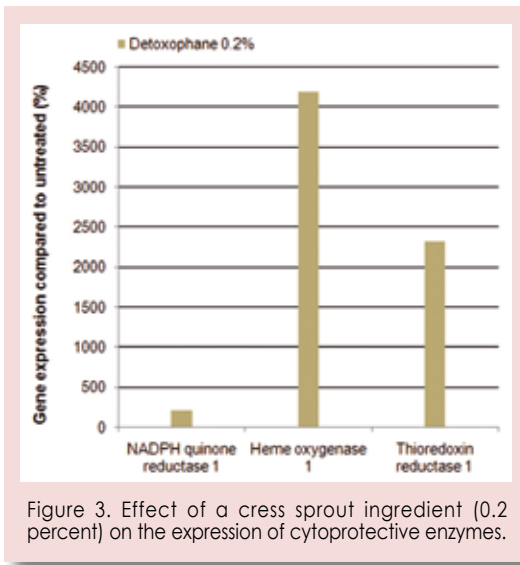


Figure 3. Effect of a cress sprout ingredient (0.2 percent) on the expression of cytoprotective enzymes.

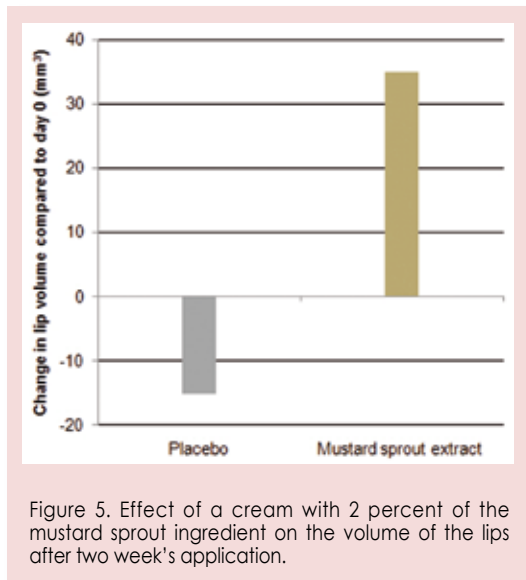


Figure 5. Effect of a cream with 2 percent of the mustard sprout ingredient on the volume of the lips after two week's application.

SUNFLOWER SPROUTS TO INCREASE THE SKIN'S CELLULAR ENERGY

Sunflower species are native in the Americas. They originate most probably from Mexico where they had been cultivated already before the Common Era. Sunflowers were planted for food and medicine but also for ornamental and ceremonial use.

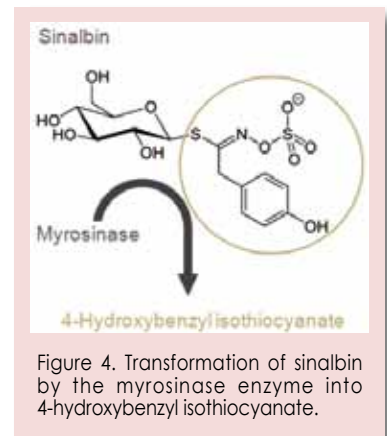


Figure 4. Transformation of sinalbin by the myrosinase enzyme into 4-hydroxybenzyl isothiocyanate.

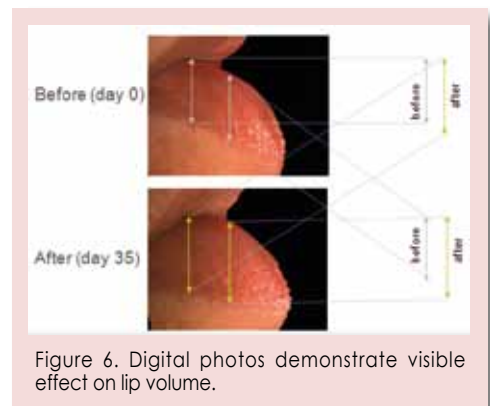


Figure 6. Digital photos demonstrate visible effect on lip volume.

European explorers brought sunflower seeds back to Europe from where they were distributed to the rest of the world. They became a rich supply of seeds for snacking and cooking. During the 18th century, the sunflower (*Helianthus annuus* L.) started being cultivated for its oil. Today, this is its primary use. Its oil is considered a premium cooking oil because of its high level of monounsaturated fatty acids.

Sunflowers turn towards the sun and symbolize power, warmth and nourishment, the attributes of the sun.

An aqueous extract of 10 days old sunflower sprouts (Sunflower Shoot Active) was tested for stimulation of cellular energy. At a cellular level, energy is produced in specialized cellular organelles, called mitochondria. There, glucose and other food molecules are oxidized to carbon dioxide and water. The energy released is stored in the form of adenosine triphosphate (ATP).

In mammalian cells, the DNA is present in the nucleus but also in mitochondria. The "mitochondrial theory of aging" states that during ATP production, in the electron transfer chain reaction, reactive oxygen species (ROS) are generated as negative side effects and that these ROS over time damage the components of the electron transfer chain and the mitochondrial DNA. As consequence, even more ROS are then generated, ending in a vicious cycle that leads to a constricted energy production. The result of limited ATP is a reduced function of cells and tissues which is aging.

The effect of the Sunflower Shoot Active ingredient on energy production was tested on reconstructed epidermis. The epidermis models were cultured in medium (control) or in medium with 2 percent of the Sunflower Shoot Active ingredient. After 4 weeks' culture, the ATP level was in general reduced compared to freshly reconstructed epidermis. Whereas the loss in the control culture accounted to 36 percent, the reduction in ATP in the epidermis cultured in the Sunflower Shoot Active medium was significantly lower, namely only 15.4 percent (Figure 7).

Using a reconstructed epidermis model we could demonstrate a decline in energy production with advancing age of the model. When the reconstructed epidermis was cultured in the presence of the Sunflower Shoot Active, deterioration in energy production could be reduced. This is a strong indication for improving skin complexion and radiance.

MATERIALS AND METHODS

Preparation of the sprout ingredients

4 to 5 days old garden cress sprouts were used as the raw material to produce Detoxophane. The INCI composition is: Lepidium Sativum Sprout Extract, Glycerin, Lecithin, Phenoxyethanol and Aqua. In the sprouts, sulforaphane is present as a 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 (8). The concentration of sulforaphane in the final ingredient was standardized at 18 mg/l. 7 days old yellow mustard sprouts were used to produce the mustard sprout ingredient. The INCI composition is: Sinapis Alba Sprout Extract, Sodium Benzoate and Aqua. 10 days old sunflower sprouts were used to produce the Sunflower Shoot Active. The INCI composition is: Helianthus Annuus Sprout Extract, Sodium Benzoate and Aqua.

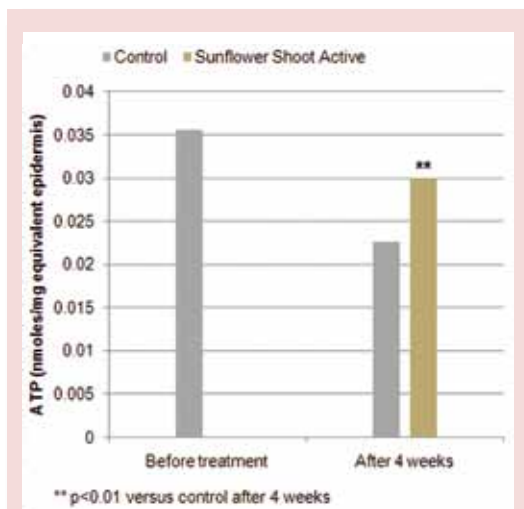


Figure 7. The ingredient Sunflower Shoot Active prevents the deterioration in energy production during cellular aging.

Stimulation of the expression of cytoprotective enzymes

The capacity of Detoxophane to modulate the expression of cytoprotective enzymes was analyzed *in vitro* using normal human epidermal keratinocytes. The method of real-time polymerase chain reaction (PCR) was used to measure the expression of the following genes: NADPH:quinone reductase 1 (NQO1), heme oxygenase 1 (HO-1), thioredoxin reductase 1 (TrxR1) and glutathione peroxidase (GPX1). The keratinocytes were grown in standard growth medium to 80 percent confluence. Then the cells were incubated for 24 hours with 0.05 or 0.2 percent cress sprout ingredient. After incubation, the cells were harvested and total RNA was extracted. The PCR reactions were performed with the LightCycler[®] system (Roche Molecular Systems Inc.). 3 replicates

per test condition were analyzed. The results were compared to the housekeeping gene liver glyceraldehyde-3-phosphate dehydrogenase.

Study on stimulation of lip volume

A cream with 2 percent of the mustard ingredient was tested on 18 women between 18 and 52 years old. First, the subjects applied twice daily over two weeks the placebo cream on their lips. After a wash out period of 1 week, the same subjects applied the cream with 2 percent of the mustard ingredient over two weeks. The volume of the lips was analyzed with the Primos Pico[®] device (Phaseshiff Rapid In vivo Measurement of Skin - GFM - Germany) and moisture of the lips with the Corneometer[®] CM 825 (COURAGE & KHAZAKA, Germany).

STUDY ON MODULATION OF CELLULAR ENERGY

The effect of the Sunflower Shoot Active ingredient on energy production was tested on reconstructed epidermis from normal human keratinocytes (SkinEthic laboratories). The reconstructed epidermis models were cultured in medium (control) or in medium with 2 percent of the Sunflower Shoot Active ingredient. After 4 weeks' culture, the ATP concentration in the epidermis models was analyzed (Biovision[®] kit). The kit uses the phosphorylation of glycerol to generate a product which is quantified by colorimetric method. The test sensibility is about 50 pmoles. For the ATP assay, 50 µl of sample were used. 50 µl of reaction mix were added to each well and the plates were incubated for 30 minutes in darkness at room temperature. Absorbance was immediately measured at 550 nm. The quantity of ATP was calculated using a standard curve. Results were expressed as nmoles of ATP / mg equivalent epidermis. The statistical analysis was done with a Fisher test.

REFERENCES AND NOTES

1. H. Schroeter, C. Heiss et al., *Mol Aspects Med.*, **31**, pp. 546-557 (2010).
2. J.K. Chavan, S.S. Kadam, *Crit Rev Food Sci Nutr.*, **28**, pp. 401-437 (1989).
3. J.W. Fahey, Y. Zhang et al., *Proc Natl Acad Sci USA*, **94**, pp. 10367-10372 (1997).
4. P. Nioi, M. McMahon et al., *Biochem J.*, **374**, pp. 337-348 (2003).
5. T. Nguyen, C.S. Yang et al., *Free Radic Biol Med.*, **37**, pp. 433-441 (2004).
6. J.L. Zhong, G.P. Edwards et al., *Photochem Photobiol Sci.*, **9**, pp. 18-24 (2010).
7. K.C. Albin, M.I. Carstens et al., *Chem Senses*, **33**, pp. 3-15 (2008).
8. Y. Zhang, K.L. Wade et al., *Anal Biochem*, **239**, pp. 160-167 (1996).