

# Shoots of sunflowers to power the skin

Sunflower Shoot Active is a cosmetic ingredient with both protective and reconstructive effects. It showed an impressive down regulation of matrix metalloproteinases in keratinocytes and protection of fibroblasts against stress-induced senescence. In reconstructed epidermis, the ingredient was found to stimulate the production of cellular energy.

Sunflower species are native to 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. 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 18<sup>th</sup> 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.

The cultivation of sunflowers is very popular because they grow easily in many types of soil. Sunflowers are known for their strong allelopathic potential. Allelopathy is the phenomenon of inhibitory or stimulatory



Figure 1: Sprouts of sunflowers.

interactions between organisms by the release of biochemicals, called allelochemicals. Sunflowers inhibit the growth of many other plant species and are researched therefore as potential natural herbicides.<sup>1</sup> Sunflower leaves have been found to produce allelopathic compounds with antibacterial effects.<sup>2</sup>

The allelochemicals of sunflower are water-soluble compounds of the phenol or terpene class of plant chemicals. These are secondary metabolites that are not required for the primary metabolic

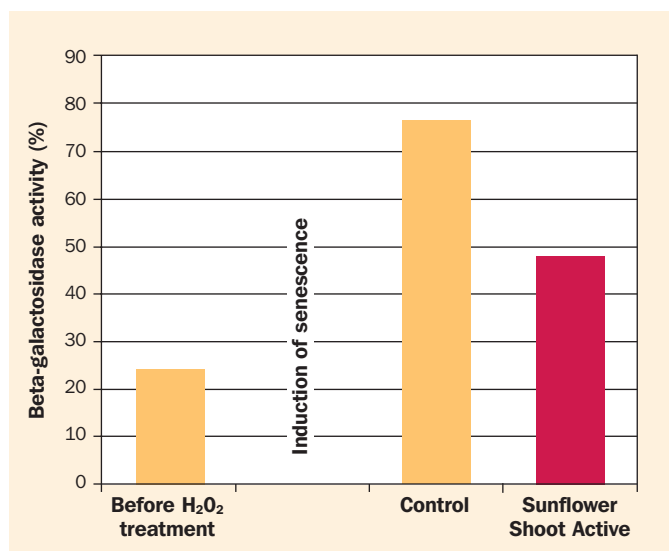
processes like growth and reproduction. The role of secondary metabolites in plants is to protect them from disease, damage, pathogens, drought, salinity, extreme ultraviolet and pollutants. Many of these phytochemicals are known to exert beneficial effects on human health or to play an active role in the amelioration of disease. Well known examples are the healthy phytochemicals of red wine grapes, the antioxidant procyanidins that help against cardiovascular diseases.

Phytochemicals are highly abundant in the shoots of a plant because at this stage plants are especially vulnerable. Shoots have higher levels of nutrients than mature plants. Plant seeds contain the embryo and stored food reserves. When under favourable conditions the seeds begin to germinate, the food reserves are mobilised. 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 forming in the growing seed, such as vitamins, enzymes and the above mentioned phytochemicals.

The sunflower shoots used to prepare the ingredient Sunflower Shoot Active are produced inside without soil (Fig. 1).

Table 1: Highly regulated genes of keratinocytes after incubation with the Sunflower Shoot Active ingredient.

Gene	Expression (Sunflower Shoot Active/Control)	Activity of Gene Product	Role/Function in Cells/Tissue
Filaggrin	2.43	Binds to keratin fibres	Skin barrier formation Skin hydration
Gamma-Glutamylcysteine Synthetase	2.27	Forms glutathione	Protection against free radicals and reactive oxygen species
Matrix Metalloproteinase 1	0.08	Cleaves collagen I, II and III	Skin thinning Loss of elasticity
Matrix Metalloproteinase 3	0.07	Degrades broad range of extracellular matrix components	Skin thinning Loss of elasticity
Matrix Metalloproteinase 9	0.23	Digests denatured collagens	Skin thinning Loss of elasticity



**Figure 2:** The ingredient Sunflower Shoot Active protects fibroblasts against stress-induced senescence.

The seeds are first soaked in water and left for two 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 four days' incubation in the containers, the shoots are harvested. Shoots are produced in the dark in order to inhibit the production of chlorophyll pigments.

An extract of sunflower shoots [Sunflower Shoot Active; INCI: Helianthus Annuus (sunflower) Sprout Extract, Sodium Benzoate] was tested in several *in vitro* systems for potential skin benefits. The high concentration of different phytochemicals in the shoots is very promising.

## Results and discussion

### Regulation of gene expression in human keratinocytes

The DNA microarray technology was used to test the effects of the Sunflower Shoot Active (now referred to as 'the new active') in an *in vitro* test with human keratinocytes. This technique uses the chip and robotics technology to analyse in one experiment the activity of hundreds to thousands of different genes. The DNA microarray is a solid support, about the size of a fingernail, onto which probes are spotted that recognise the activity of their corresponding genes. For this experiment the PIQOR Skin Microarray of Miltenyi Biotec GmbH was used. The array contains 1308 genes that are known markers for skin barrier, extracellular matrix, DNA repair, signalling, detoxification, inflammation, skin cancer and other skin disorders.

Normal human keratinocytes were incubated for 24 hours with the new active. In parallel, a control culture was incubated

in the same culture medium without the ingredient. A sample of both cultures was then analysed on the DNA microarray. The results of the gene array are summarised in Table 1. Only genes that are highly up-regulated (>1.9) or highly down-regulated (<0.4) in presence of the new active were taken into account for the efficacy evaluation.

The highly up-regulated genes code for the protein, filaggrin, and the enzyme, gamma-glutamylcysteine synthetase. Filaggrin, a marker for epidermal differentiation, is very important for the maturation of the *stratum corneum*. Filaggrin is used for the formation of the keratin network and provides free amino acids that serve as natural moisturising agents. Loss of filaggrin leads to a poorly formed *stratum corneum* which is prone to water loss.<sup>3</sup> Stimulation of filaggrin production improves the skin barrier. Gamma-glutamylcysteine synthetase is the enzyme used for the synthesis of glutathione, a very important cellular antioxidant. Glutathione is a tripeptide with the sulfhydryl group of cysteine as the active site. In the reduced state, the sulfhydryl group reduces unstable reactive oxygen species. The stimulation of the synthesis of glutathione will enhance the defence against reactive oxygen species and free radicals.

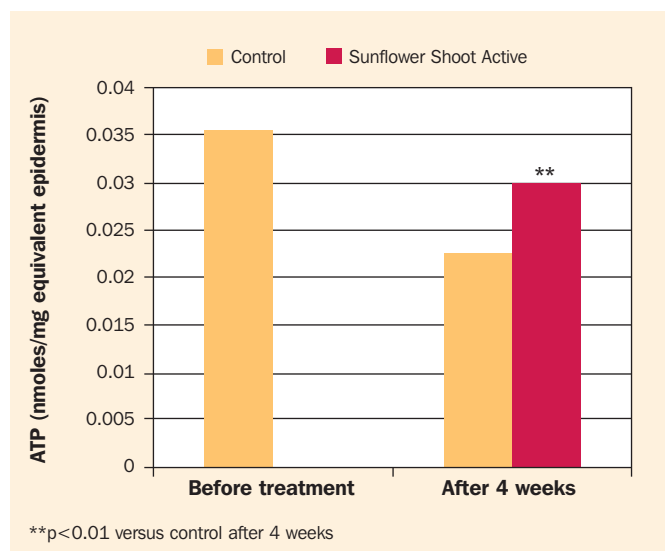
The most down-regulated genes encode three enzymes of the same family, the matrix metalloproteinase family that comprises enzymes responsible for degradation of the extracellular matrix. Matrix metalloproteinases play an important role in the metabolism of matrix components such as collagen and elastin and are essential, for example, for healing wounds. But it is also known that they are predominant in elderly skin, leading to an

imbalance in synthesis and degradation of matrix components. The skin loses its firmness and elasticity. Matrix metalloproteinases are also involved in photoageing of the skin. Exposure of skin cells to ultraviolet irradiation leads to increased expression of matrix metalloproteinase genes.<sup>4</sup> Inhibition of the expression of matrix metalloproteinases, or of their enzymatic activity, is the mechanism of action of many actives against skin ageing. The results in Table 1 show a drastic down-regulation of matrix metalloproteinase genes in keratinocytes after incubation with the new active. They prove a strong protection/anti-ageing efficacy of the new active ingredient.

### Prevention of premature senescence in human fibroblasts

On human skin fibroblast cells, the new active ingredient was tested for protection against stress-induced premature senescence. Cellular senescence is an arrest in cell proliferation, induced by damaged DNA. It prevents tumoral growth. But cellular senescence is also thought to contribute to ageing. Impaired cell mobility and secretion of matrix-degrading enzymes and of pro-inflammatory cytokines are associated with senescence.<sup>5</sup> Accumulation of senescent fibroblasts will lead to severe skin damage.<sup>6</sup> It is therefore very important to prevent premature senescence.

In the study with the new active, premature senescence in fibroblasts was induced by incubating the cells in H<sub>2</sub>O<sub>2</sub> for two hours. Cells were then cultured in medium (control) or in medium with 2% of the new active ingredient. After six days' culture, the fibroblast cells were analysed for senescence with the beta-galactosidase assay. Beta-galactosidase is a senescent phenotype-associated marker that can be



**Figure 3:** The ingredient Sunflower Shoot Active prevents the deterioration in energy production during cellular ageing.

detected by histochemical staining. Compared to the control culture, addition of 2% of the new active resulted in a 55% protection against premature senescence (Fig. 2). The exact mechanism is not known, but it could be that the ingredient supported the DNA repair processes.

#### Stimulation of cellular energy in 3D epidermis

At a cellular level, energy is produced in specialised cellular organelles, called mitochondria. There, glucose and other food molecules are oxidised 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 ageing" 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 (mtDNA). As a 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 ageing.

The effect of the new 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% of the new active ingredient. After four weeks' culture, the ATP concentration in the epidermis models was analysed (Biovision kit). The ATP level after four weeks was in general reduced compared to freshly reconstructed epidermis (Fig. 3; start). But the epidermis cultured in the new active medium contained significantly more ATP than the epidermis of the control culture.

#### Conclusion

The results of the different skin cell culture assays presented here proved an explicit anti-ageing effect of the ingredient Sunflower Shoot Active. 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. **PC**

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