PRODUCT FOCUS

How to tackle uneven pigmentation

INFLUENCE OF UNEVEN SKIN TONE ON PERCEPTION OF BEAUTY

Blotchy skin and so-called age spots cause an uneven skin tone which is a major concern for women, equal to dry skin and wrinkles. Age spots, also known as sun- or liver spots, are brown to black macules that are common on sun-exposed skin after the age of 40. Age spots are harmless and do not really need treatment, but they are a significant visual clue to a woman's age. This was clearly shown in a study where digital imaging technology was used to standardize female faces in form and surface topography. Rating of a set of standardized faces, varying only in age- and photo damage-induced skin colour distribution, revealed that age spots have a major influence on the perception of female facial age (1).

THE DRAWBACK OF THE TYPICALLY USED WHITENING INGREDIENTS

The cosmetic treatment of age spots is normally a combination of the regular use of sunscreens for prevention and application of whitening products to fade the spots. Chemical peels are another option. But, if not applied only to the spot area, the fading effect is minimal because bleaching creams and peelings will whiten the skin all over. And as most of the women still prefer a slight, healthy-looking tan, they are looking for a product that specifically treats age spots. This article describes a cosmetic ingredient that meets these criteria. The ingredient is based on a combination of a cress sprouts extract with the soy isoflavone genistein.

REGULATION OF PIGMENTATION IN AGE SPOTS

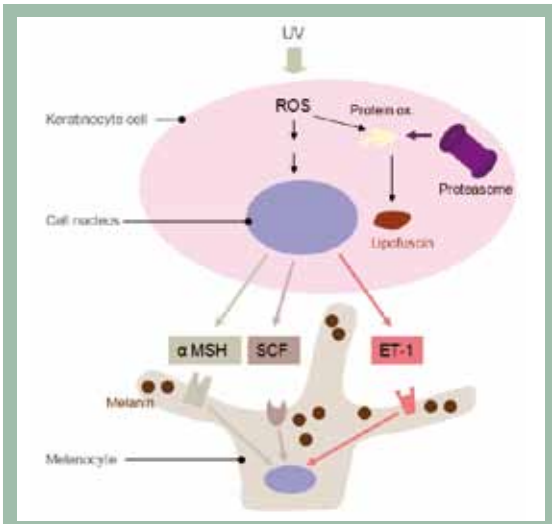


Figure 1. Signalling mediators involved in the formation of lipofuscin and melanin in age spots.

Two types of pigments are present in age spots, the melanins and lipofuscin. They are overproduced in the spot area because of an overreaction to UV radiation. UV light leads to the generation of free radicals and reactive oxygen species in keratinocytes. This induces the formation of signalling molecules like the α-melanocyte stimulating hormone (α-MSH), the endothelin-1 (ET-1) and the stem cell factor (SCF), and leads also to the oxidation of proteins and lipids (Figure 1). Oxidized proteins and lipids form insoluble, dark pigmented complexes, called lipofuscin. The released signalling molecules bind to their corresponding receptors on melanocytes where they stimulate melanin production and promote dendrite formation. The messenger molecules ET-1 and SCF are shown to be greatly overproduced in age spot areas (2). This explains the higher melanin content compared to the neighbouring, normally pigmented skin. Accumulation of oxidized proteins and thus lipofuscin formation is normally prevented by the proteasome system. It is a complex of proteases that specifically recognizes damaged proteins and then degrades them completely. But proteasome activity is known to decline with advancing age (3), explaining why uneven pigmentation is a typical symptom of old age.

A NEW, TARGETED TREATMENT OF AGE SPOTS

The cress sprouts extract helps in several ways against age spots:

1. It contains isothiocyanates that work as indirect antioxidants;
2. It has a general whitening effect by inhibiting the α-MSH-induced melanin synthesis;
3. It has a specific effect on age spots by stimulating the proteasome system.

Cress sprouts are a rich source of the isothiocyanate sulforaphane. Isothiocyanates are sulfur-containing chemicals that are characteristic of the Brassicaceae family. Well known members of this family include broccoli, rapeseed, mustard, radish and cress. Isothiocyanates give these vegetables their typical pungent taste and are produced to repel herbivores. Sulforaphane works as an indirect antioxidant (4). Direct antioxidants such as the vitamins C and E can neutralize an oxidant once and need then to be replenished by other antioxidants.

Indirect antioxidants work by expressing a series of genes that code for cytoprotective proteins. These proteins are enzymes that synthesize or regenerate a lot of different direct antioxidants. Enzymes work catalytically, meaning that they are able to do thousands of working steps without being consumed. In this way, sulforaphane can be used to neutralize free radicals and reactive oxygen species that represent the first UV-induced triggers for the formation of lipofuscin and melanins.

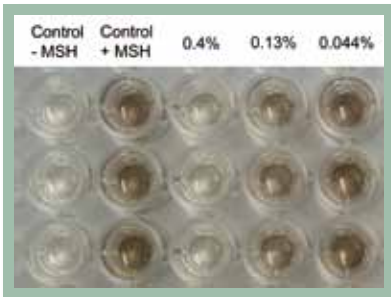


Figure 2. Incubation plate with the B16 murine melanocytes in triplicates.

The cress sprouts extract was also found to inhibit the a-MSH-induced melanin synthesis. This was shown 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 a-MSH. After incubation, the melanin content was analyzed by measuring the optical density at 405 nm. A plate that was cultivated in parallel was used for the evaluation of cell viability by the MTT assay. The cress sprouts extract was tested at three different concentrations. Melanin formation was strongly inhibited at 0.4 percent (Figure 2). The MTT assay clearly demonstrated that this was not the consequence of a cytotoxic effect. The inhibitory effect of the extract on melanin formation after stimulation with a-MSH could also be demonstrated with normal human melanocytes. The cress sprouts extract at 0.016 percent reduced melanin synthesis by 47 percent. The cress sprouts extract was not active in assays with isolated human tyrosinase. The results of the cell-based assays with B16 cells and the normal human melanocytes therefore indicate that the cress sprouts extract reduces the binding of a-MSH to its receptor on melanocytes.

The cress sprouts extract turned out to be very active in a cell-based screening assay for modulators of the proteasome system. The Proteasome-Glo™ Assay (Promega) was used to analyze the effects on the proteasome activity in normal human dermal fibroblast cells. The assay is based on a proteasome substrate labeled with aminoluciferin. This coupled-enzyme system, with simultaneous proteasome cleavage of substrate and luciferase consumption of the released aminoluciferin, results in a luminescent signal that is proportional to the proteasome activity. Figure 3 shows that the proteasome activity in young fibroblasts (passage 8) was 15 percent higher than in old cells (passage 16). This result corresponds to the age-related decline in the proteasome activity (3). Four hours after incubation with 0.33 percent of the cress sprouts extract, the proteasome activity of old fibroblasts was increased by 71 percent. By stimulating the proteasome activity, the cress sprouts extract can prevent or reduce the accumulation of oxidized proteins and thus inhibit lipofuscin formation.

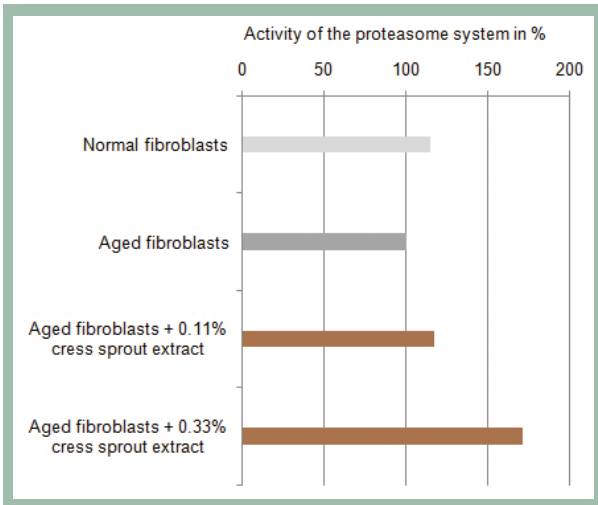


Figure 3. Stimulatory effect of the cress sprouts extract on the proteasome activity.

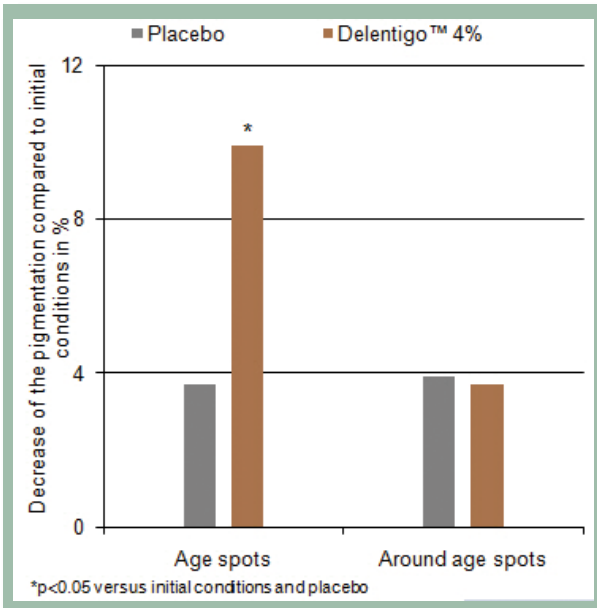
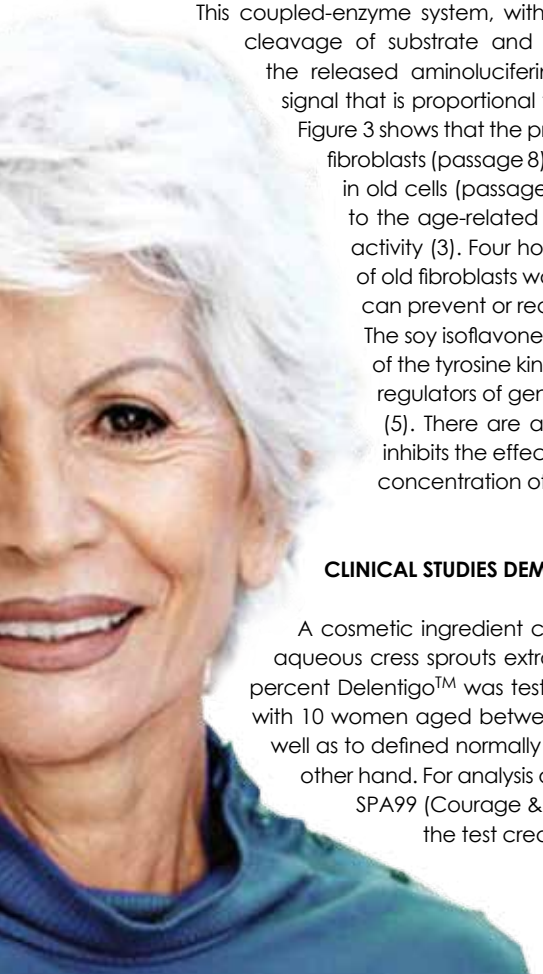


Figure 4. The effects of the test creams on melanin index after four weeks' application.

The soy isoflavone genistein is also specifically working on age spots. Genistein is a well known natural inhibitor of the tyrosine kinase, an enzyme involved in several signalling cascades from receptors at the cell surface to regulators of gene expression. A tyrosine kinase is reported to be at the intracellular side of the SCF receptor (5). There are also reports about the involvement of tyrosine kinase in the ET-1 signalling. Thus, genistein inhibits the effects of SCF and probably also of ET-1 on melanocytes. In this way, genistein regulates the high concentration of SCF and ET-1 typically found in age spots.



CLINICAL STUDIES DEMONSTRATE THE EFFICACY IN AGE SPOT TREATMENT

A cosmetic ingredient containing 1.4 g/kg genistein, encapsulated into lecithin liposomes, and 37 percent of an aqueous cress sprouts extract, standardized to 100 µM sulforaphane was developed (Delentigo™). A cream with 4 percent Delentigo™ was tested in two clinical studies. The first double-blind, placebo-controlled study was performed with 10 women aged between 47 and 77. The test cream was applied twice daily for four weeks to defined spots as well as to defined normally pigmented skin areas on one hand. The placebo cream was applied in similar way to the other hand. For analysis of skin pigmentation, the melanin index was measured with the Skin Pigmentation Analyzer® SPA99 (Courage & Khazaka) at the beginning of the study and after four weeks. The study results showed that the test cream could significantly fade the age spots (Figure 4).

After four weeks' application and compared to age spots treated with the placebo cream, the melanin index was reduced by 6.2 percent. There was no difference in the melanin index in the normally pigmented skin between the test cream and the placebo.

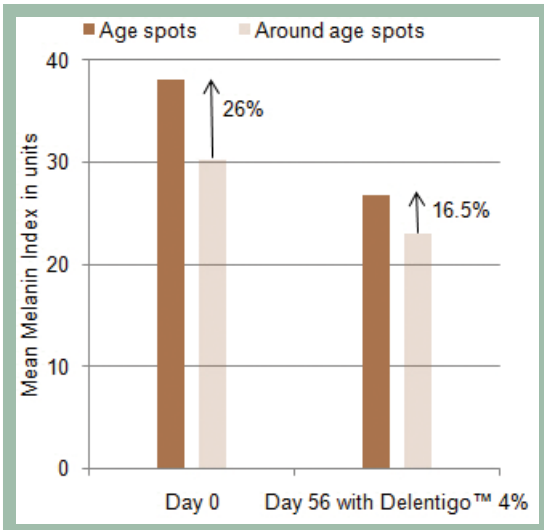


Figure 5. The effect of the test cream on melanin index after eight weeks' application.

The second study was also double-blind, placebo-controlled, but carried out over 8 weeks and was conducted on 12 volunteers. The same treatment conditions were applied. After 8 weeks' application, the bleaching effect was clearly more pronounced and also measurable in the normally pigmented skin areas (Figure 5). But it was again possible to demonstrate a better effect of the test cream on the spot zone compared to the neighbouring, normally pigmented skin. The 26 percent higher melanin index of the age spot, measured before application, could be reduced to a difference of only 17 percent after 8 weeks' treatment. Also on digital photos one could easily see that the age spots were less prominent after the treatment (Figure 6).

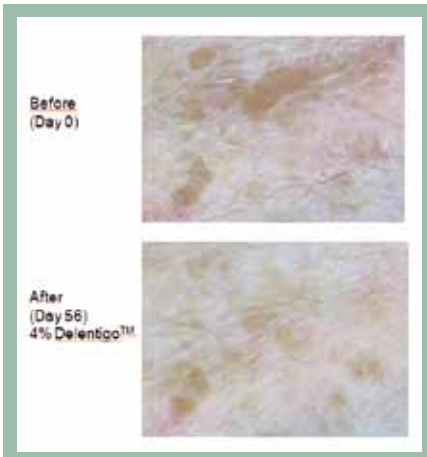


Figure 6. Photos of age spots on the hand of one subject, before and after treatment.

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