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Genistein, a new cosmetic ingredient derived from soy

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Introduction

Health benefits of soy isoflavones

Epidemiological studies have demonstrated that the Asian diet is closely linked with a lower incidence of certain cancers, cardiovascular diseases and postmenopausal symptoms. Meanwhile it is well accepted that these effects are based on isoflavones from soybeans. Therefore a large number of functional food products, containing soy isoflavones as active ingredient, have been launched in the last few years. Isoflavones are non-nutrient, bioactive molecules of a polyphenolic structure. As they resemble the human hormone estrogen they are also classified as phytoestrogens.

Soybeans primarily contain the isoflavones genistein and daidzein and their respective β-glycosides, genistin and daidzin. In nonfermented soyfoods, they occur predominantly in form of the polar, water-soluble glycosides (*Wang* and *Murphy* 1994). However, the bioactive form is the sugar-free isoflavone which is called aglycone (*Setchell* 1998). Thus the natural soy isoflavones have to be activated to become functional through the hydrolysis of the sugar moiety **(Fig. 1)**.

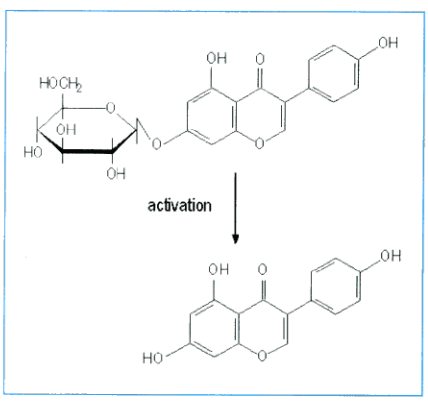

Fig. 1 Transformation of the glycoside genistin to the bioactive aglycone, genistein

Isoflavones as phytoestrogens: against postmenopausal skin ageing

Menopause and skin ageing

During normal ageing, the skin becomes thinner, looser and less elastic. In men, this is a fairly gradual process. In women however, menopause causes severe changes in the skin as a result of the drastic drop in the production of the ovarian hormones estrogen and progesterone. During menopause, we often see skin dryness, wrinkling, fragility, bruising, and even acne. The histologic analysis of aged skin shows more profound alterations in the dermis than in the overlaying epidermis **(Fig. 2)**. The dermis is composed of fibrillar collagen bundles and elastic fibers in a complex array of proteoglycans and other extracellular matrix components. Fibroblast cells are embedded within the matrix. The proteins collagen and elastin impart strength and resiliency to the skin. Histologically skin ageing is associated with a profound atrophy of the dermal connective tissue (*Campisi* 1998).

In human skin, estrogen receptors have been identified (*Hasselquist* et al. 1980). Thus estrogen plays a very important role in skin health and skin ageing. Estrogen stimulates the production of collagen and elastin (*Brincat* et al. 1987, *Affinito* et al. 1999) and on the other hand inhibits the breakdown of the existing collagen. Falling estrogen levels result therefore in laxity of the skin and a decreased general skin tone which leads to sagging and wrinkles.

Hormone replacement therapies are widely used to relieve post menopausal symptoms, to reduce the risk of osteoporosis and heart attacks, and to prevent hormone related skin ageing. Several studies show that estrogen therapy preserves collagen content, elastic properties, and thickness of the skin in women after menopause (*Callens* et al. 1996, *Schmidt* et al. 1996, *Dunn* et al. 1997). Hormones are applicated by two routes, either oral or

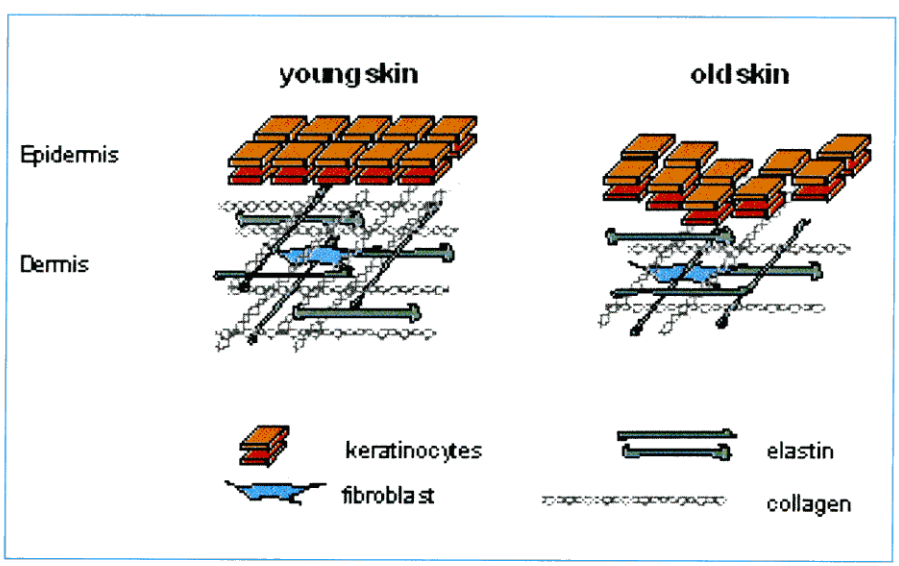

Fig. 2 Schematic drawing of young and old skin

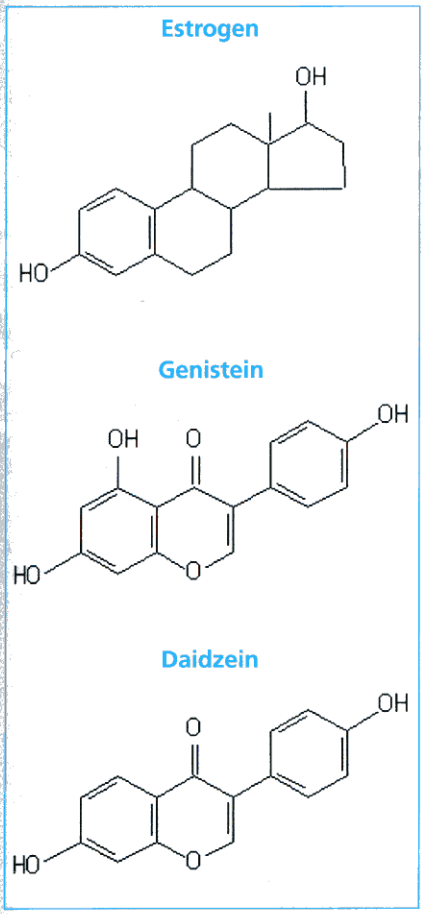

Fig. 3 Molecular structures of the hormone estrogen and the phytoestrogens genistein and daidzein

topical. Because of better effects, the transdermal route to deliver hormones is nowadays preferred by pharmaceutical companies. Hormones penetrate very well through the skin, while most of the hormones are degraded before they reach the blood system on the oral route. Today, hormone replacement therapies with estrogen are highly debated because they seem to induce an increased risk of breast and uterus cancer as long-term side effect.

The phytoestrogen genistein for an alternative hormone replacement therapy
Isoflavone aglycones are structurally very similar to steroidal estrogens **(Fig. 3)** and can therefore bind to some extent to the same receptors. In human, two different estrogen receptors, ER α and ER β exist. Compared with the human estrogen, genistein has a much lower affinity to ER α but about the same affinity to ER β (*Kuiper* et al. 1998). Genistein exerts a tissue-dependent estrogenic effect. It is especially active in bones and the cardiovascular system and less in breast and uterus (*Cassidy* 1999, *Maroulis* 2000).

The application of hormones in cosmetic products is not allowed. Phytoestrogens, like isoflavones, can be used as an ideal, natural alternative to hormones for the prevention of postmenopausal skin ageing. Contrary to the human hormone estrogen, soy isoflavones do not show adverse side effects. Studies show that soy isoflavones can even reduce the incidence of certain cancers (*Setchell* 1998). Once the sugar moiety is hydrolysed, isoflavones become lipophilic, bioactive compounds, that like estrogen will penetrate well into the skin. If the skin is the place of action for a given functional ingredient, the transdermal pathway represents the shortest delivery route. Application of isoflavones in a cosmetic product is therefore the ideal approach for the prevention of skin ageing.

Isoflavones as kinase inhibitors: a new cosmetic ingredient for anti-ageing products
Genistein also shows several estrogen-unrelated activities (*Knight* and *Eden* 1996). It inhibits for example the enzymes tyrosine protein kinase and MAP kinase. These enzymes are involved in the transmission of intracellular signals that modulate cellular growth and differentiation. These cellular processes regulate the function of tissues, for example of the skin.
Cell culture assays showed that genistein regulates the metabolism of the skin matrix components collagen and elastin. Reduction in these components and therefore shrinking of the dermal matrix is the basic mechanism of skin ageing **(Fig. 2)**. As a tyrosine protein kinase inhibitor genistein stimulates the de novo production of colla-

gen (*Yoon* et al. 1998), inhibits the expression of proteinases which specifically degrade collagen or elastin, the so called matrix metalloproteinases, and stimulates at the same time the expression of tissue inhibitors of metalloproteinases, the so called TIMP (*Shao* et al. 1998, *Ravanti* et al. 1999, *Kim* et al. 2001). Overall genistein prevents by this estrogen-independent mechanism the degradation of the dermal matrix and counteracts thereby the principal skin ageing mechanism.

Isoflavones as lipolytic agents: an active compound in the treatment of cellulite

About cellulite
Cellulite is usually located in the buttocks and thigh regions and is characterised by dimpling and pitting skin. There are principally gender-related differences in adipose and connective tissue which favour the development of cellulite in women versus men. Female connective tissue allows external expansion of adipose tissue into the dermis and therefore favours dimpling. If the connective tissue which separates the dermal and the adipose tissue layers is inherently weaker or becomes progressively thinner and looser as a woman ages then the adipose tissue starts to extrude outwards into the dermis, a process manifested as cellulite.

Properties of genistein that contribute to its anti-cellulite activity
Genistein was found to modulate growth and differentiation of adipose tissue and to interfere with lipid metabolism, activities that are linked with the development of cellulite. Genistein inhibits the proliferation of preadipocytes (*Harmon* and *Harp* 2001) and it inhibits the enzyme phosphodiesterase thereby increasing lipolysis (*Kuppusamy* and *Das* 1992, *Szkudelska* et al. 2000). Genistein is known to stimulate epinephrine-induced lipolysis (*Nogowski* et al. 1998) and to inhibit insulin-induced lipid synthesis (*Nogowski* et al. 1998). Overall these activities lead to the reduction of adipose tissue. Further, the estrogen-related as well as protein kinase-related effects of genistein on the connective tissue proteins collagen and elastin results in a reinforcement of the connective tissue and the dermis. Thereby the tendency of adipose tissue to expande outwards is reduced.

Results

Development of an anti-cellulite preparation
For our anti-cellulite study, genistein was mixed with two well-known anti-cellulite compounds, caffeine and carnitine, and with a hydrophilic extract of *Spirulina platensis*. Caffeine as methylxanthine derivative inhibits the enzyme phosphodiesterase thereby increasing lipolysis in adipose tissue (*Scotini* et al. 1983). Carnitine is a cosubstrate for the translocation of long-chain fatty acids in mitochondria and thus a stimulator of lipid oxidation. The cyanobacterium *Spirulina platensis*, a filamentous blue-green algae, is a rich source for unsaturated fatty acids, essential amino acids, minerals, carotenoids and polysaccharides. The extract of *Spirulina* cells offers many interesting effects in topic applications. The polysaccharide spirulan is a good skin moisturizer and might also stimulate lipolysis as it was reported for polysaccharides of brown sea algae (*Rozkin* et al 2000). The carotenoids protect the skin against oxidative stress.

In vivo activity of the anti-cellulite preparation

Study design
In the study, the right thigh of 20 women between age 36 and 57 with light to heavy cellulite was treated once daily with a cream containing the anti-cellulite preparation **(Table 1)**. The other thigh stayed untreated and served as control. After 3 and 6 weeks of application, the different skin parameters were measured and compared to the starting values. The study was conducted at Derma Consult GmbH, Germany.

Ingredient	Concentration (%)
Genistein	0.006
Caffeine	0.03
Carnitine	0.15
Spirulina platensis extract	0.048

Table 1 Concentration of single ingredients in the anti-cellulite cream

Skin parameters
Measurement of skin roughness: Determination of skin roughness was carried out with the optical 3D-in-vivo-skin measurement system »Primos« (Phase-Shifting Rapid in vivo Measurement of skin / GFMesstechnik GmbH,

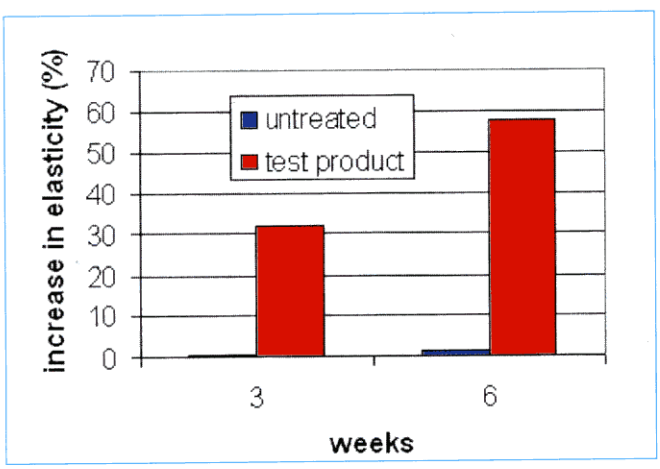

Fig. 4 Improvement of skin elasticity by the anti-cellulite cream

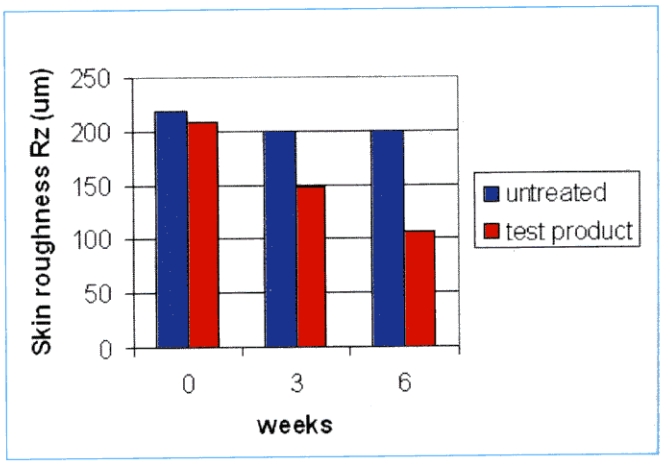

Fig. 5 Reduction of skin roughness (smoothing effect) by the anti-cellulite cream

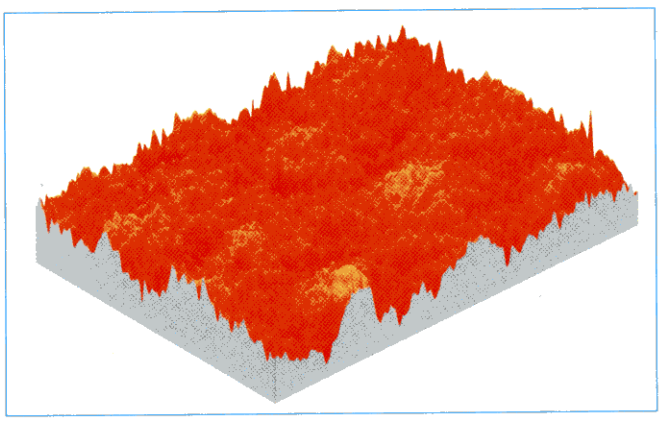

Fig. 6 a Three-dimensional visulisation of skin roughness data points taken before treatment with the anti-cellulite cream

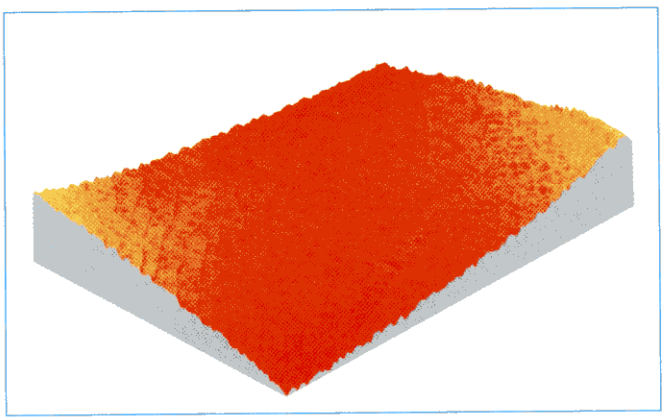

Fig. 6 b Three-dimensional visualisation of skin roughness data points taken after six weeks treatment with the anti-cellulite cream

14513 Teltow / Berlin) which allows fast three-dimensional in vivo measurement of the microtopography of human skin based on the technology of active image triangulation.
Measurement of skin elasticity: Skin elasticity was measured by means of Cutometer SEM 474 (Courage&Khazaka Electronic GmbH, Cologne).
Measurement of thigh girth: The diameter was measured about mid-way between the knee an the hip.
Determination of cellulite grading: Trained evaluators graded the level of cellulite symptoms using a 5-point scale (0-4). 0 = no cellulite; 1 = slight dimpling of the skin surface; 2 = dimpling and skin depressions; 3 = dimpling and depressed striations; 4 = palpable nodules and striations.

Study results
Fig. 4 shows that the application of the anti-cellulite cream, containing genistein, caffeine, carnitine, and *Spirulina platensis* extract, significantly increased skin elasticity in a timely manner and reached almost 60 % improvement after 6 weeks treatment compared to the starting value. At the sime time the skin roughness was reduced by about 50 % **(Fig. 5)**. This smoothing effect is nicely demonstrated by 3-dimensional visualisation of the skin roughness data points **(Fig. 6a and b)**. The cellulite degree dropped from 3.7 at the start of the study to a value of 2.3 after 6 weeks. This remarkable improvement in the visible signes of cellulite was confirmed by a clear mean reduction of the thigh girth, that was 2 cm after 6 weeks.

Conclusion

Soy isoflavones are already widely used as functional food ingredients. In most cases, these food supplements contain only isoflavone glycosides, the molecular form that is biologically not active. However, after ingestion the glycosides are transformed by intestinal glycosidases and intestinal bacterial metabolism into the estrogenically active form (*Setchell* and *Cassidy* 1999). Since the skin does not harbour such bacteria and enzymes, the active isoflavone preparations for skin care must be in the form of aglycones. Unfortunately, these aglycones have a poor solubility in water and oil. Thus, a special galenic form is necessary to introduce these isoflavone preparations into cosmetic formulations.
We developed a soy isoflavone preparation suitable for cosmetic applications. It contains the biologically active aglycone genistein in pure form (*Schmid* et al. 2001). Our genistein formulation is based on liposomes to make the aglycone water dispersible and bioavailable to the skin.

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