Dermatological Application of Soy Isoflavones to Prevent Skin Ageing in Postmenopausal Women

Schmid Daniel, Muggli Reto and Zülli Fred
Mibelle AG Cosmetics, Bolimattstrasse 1, 5033 Buchs, Switzerland

Abstract

Isoflavones from soy beans are used in functional food products as a measure to alleviate hormone-dependent physiological disorders such as postmenopausal symptoms, osteoporosis and cardiovascular problems. These natural phytohormones are well perceived and do not show the negative side effects found in conventional therapies with hormones such as estrogens. A number of studies show that estrogens can slow down the aging process of the skin in postmenopausal women. For this reason, isoflavones from soy beans are also very interesting compounds for cosmetic formulations. We succeeded in isolating very pure fractions of isoflavones in the form of the active aglycones suitable for cosmetic applications. We herein report the use of this aglycone preparation in the treatment of post menopause skin and discuss further applications.

Phytoestrogens are plant derived compounds with biological activities comparable to the human hormone estrogen

Epidemiological studies indicating associations between diet and disease states led to the investigation of a series of bioactive plant compounds, called phytochemicals. Some of these nonnutrient plant chemicals, such as phytoestrogens were shown to confer significant long-term health benefits. Phytoestrogens are structurally and functionally similar to human estrogen (Fig. 1). There are two types of phytoestrogens, the lignans and the isoflavones. Soybeans are especially rich in isoflavones. They contain the isoflavones genistein and daidzein and their respective β-glycosides, genistin and daidzin (Fig. 1 and 2). In nonfermented soyfoods, they occur predominantly in form of the polar, water-soluble glycosides, whereas fermented soy products contain mainly the biologically active aglycones (Wang and Murphy 1994).

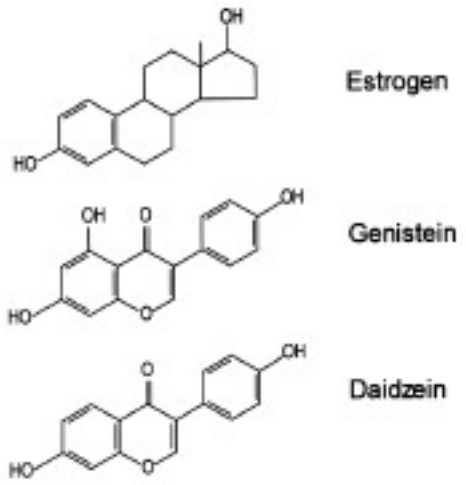
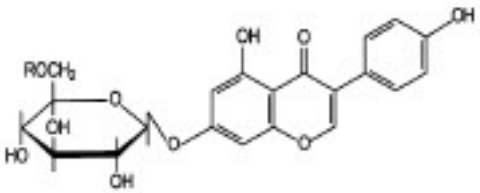


Fig. 1. Molecular structures of the hormone estrogen and the phytoestrogens genistein and daidzein



R	Compound
H	Genistin
$COCH_3$	Acetylgenistin
$COCH_2$	Malonylgenistin

Fig. 2. Molecular structures of the isoflavone glycosides of genistin

The role of the hormone estrogen and the mechanism of its action

Estrogen influences growth, differentiation and function of the female and male reproductive system such as mammary gland, uterus, vagina, ovary, testes, epididymis, and prostate as well as other tissues (*Korach et al. 1995*). Estrogen plays an important role in the development and maintenance of the bone structure as well as for the cardiovascular system (*Turner et al. 1994*, *Farhat et al. 1996*). The three principal forms of estrogens, which are produced mainly in the ovaries and testis, are 17β-estradiol, the most active form, estrone and estriol. Upon arrival at the target cell the estrogens are retained by intracellular binding proteins, the estrogen receptors (ER). The saturated ER undergoes a conformational change that allows the receptor to interact with the DNA and to modulate the transcription of target genes (*Jensen 1995*).

Estrogen-related physiological disorders and their therapy

As the ovaries age, their function declines until menopause where the production of estrogen and progesterone drops drastically. These hormonal changes lead to menopausal symptoms like hot flashes which have a severe negative impact on many women's quality of life. In addition, they are considered to be a potential risk factor for osteoporosis and cardiovascular diseases (*Utian 1989*). Hormone replacement therapies have been found to help against these menopause-related disorders. However, the continuous administration of estrogen is sometimes associated with severe adverse effects such as vaginal bleeding and in rare cases with the development of breast and uterus cancer (*Breckwoldt 1995*). Synthetic estrogen-like compounds, called selective estrogen receptor modulators (e.g. raloxifene®), were introduced recently for an improved hormone replacement therapy (*Compston 1998*). The perfect drug should have a tissue selectivity and exert an estrogenic effect only in bones to prevent osteoporosis and in the cardiovascular system. Thus the compound should act as a non- or anti-estrogen in breast and uterus (Table 1). Recently a second estrogen receptor (ERβ) was found in addition to the classical (ERα) one. The tissue distribution and the ligand binding affinities of the two receptors are different (*Kuiper et al. 1997*). The effectiveness of selective estrogen receptor modulators depends on their uptake by the various tissues and on their specific affinities to the two estrogen receptors.

Phytoestrogens as natural alternative to estrogen replacement therapies

Isoflavones are currently intensively studied as natural alternatives to human estrogens used in replacement therapies. The fact that Asian women, that traditionally consume a lot of soy products, are less affected by the menopausal symptoms, such as osteoporosis, cardiovascular disease, and breast cancer, is attributed to their high isoflavone intake (*Adlercreutz et al. 1992*). Food products supplemented with soy isoflavones - so called 'functional foods' or nutraceuticals - are claimed to prevent postmenopausal problems, especially osteoporosis. The structures of isoflavones, heterocyclic phenols, are very similar to the steroidal estrogen (see Fig. 1). Thus they can bind to some extent to estrogen receptors.

Compared with the principal circulating estrogens in humans, isoflavones are bound at a much lower rate (10^3-fold weaker), however, they do compete with estrogens for receptor sites. Recent data show that phytoestrogens have higher affinities to ERβ than to ERα. Phytoestrogens have been shown to protect against osteoporosis and heart disease without affecting the uterus and breast (*Kuiper et al. 1998*, *Cassidy 1999*). Clinically, isoflavones may behave like selective estrogen receptor modulators. Thus, the application of isoflavones may have decisive advantages over classical hormone replacement therapies.

Table 1. Selective tissue effects of synthetic estrogen-like compounds (e.g. raloxifene) and isoflavones (Maroulis 2000).

Target Tissue	*Brain*	*Uterus*	*Breast*	*Bone*	*Cardiovascular*	
Compound						
Estrogen	+ +	+ +	+ +	+ +	+ +	
Raloxifene		—	—	—	+ +	+ +
Isoflavones		+	—	—	+	+

Benefits of phytoestrogens in skin care

After menopause, the skin undergoes changes that include thinning, decrease in elasticity and wrinkle formation *(Bolognia et al. 1989)*. This abrupt skin aging is the consequence of a lower production of collagen and elastin, the supportive and elastic proteins of the skin (*Brincat et al. 1987, Affinito et al. 1999, Adamiak et al. 2000*). Thus, the skin appears to be an organ responding to ovarian hormones. Indeed estrogen receptors have been identified in human skin (*Hasselquist et al. 1980, Schmidt et al. 1990*). The question as to how far this skin aging is induced by postmenopausal hypoestrogenism is still under discussion (*Affinito et al. 1999*).

Several studies show that estrogen therapy preserves collagen content, elastic properties, and thickness of the skin in postmenopausal women (*Hasselquist et al. 1980, Punnonen et al. 1987, Castelo Branco et al. 1992, Dunn et al. 1997*). Topical estrogen treatments were shown to reverse postmenopausal skin aging (*Callens et al. 1996, Schmidt et al. 1996*). Estrogenic compounds reduce the depth of wrinkles and increase the tone and hydration of the skin.

Since the results obtained with isoflavone supplemented nutraceuticals are very positive, we believe that the topical application of phytohormones can also fight some of the menopause-associated skin problems. In *in vitro* testing genistein stimulated collagen synthesis (*Kawashima et al. 1996*) and down regulated matrix metalloproteinases (*Shao et al.*). Therefore topical application of isoflavones is not only beneficial in postmenopausal skin, where it replenishes the loss of endogenous estrogen but also in normal skin when used to treat cellulite or dull skin in general.

Development of a pure isoflavone preparation suitable for skin care

There are several soy isoflavone products on the market that are sold as dietary food supplements. 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 glucosidases and intestinal bacterial metabolism into the estrogenically active form (*Setchell and Cassidy 1999*). Since the skin does not habour such bacteria and enzymes, the active isoflavone preparations for skin care must be in the form of aclycones. 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 process to prepare a pure isoflavones suitable for cosmetic applications (Fig. 3).

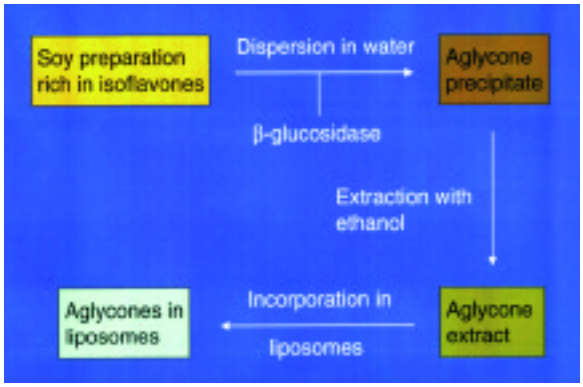


Fig. 3. Production process for a cosmetic phytoestrogen ingredient

A soy preparation which is rich in isoflavones is treated with a βglucosidase to deglycosylate the isolflavones to the corresponding aglycones. As a consequence of their hydrophobic nature, these aglycones precipitate almost entirely and can be separated by centrifugation from the enzyme and the water-soluble brown polyphenols. The aglycones are extracted with alcohol to get a phytohormone solution. The aglycones can then be incorporated into liposomes to get a water dispersable preparation which has a high bioavailability in the skin.

Analytical characterization of isoflavones

Isoflavones have an absorption maximum at 260 nm and can be separated on a reverse phase column. Therefore the aglycone formation in our purification process can be followed by HPLC analysis. The analysis of the starting material revealed that the isoflavone glycosides daidzin and genistin are the main components. After the β-glucosidase treatment, these glycosides were completely transformed to their corresponding aglycones daidzein and genistein (Fig. 4). The Genistin of the source material could be recovered at 86% as genistein and daidzin at 50% as daidzein respectively after alcohol extraction. The characterization of the aglycone solution in alcohol revealed that the product has a purity of > 80%. Upon dilution of this alcohol solution in water white needles of geinstein and daizein became visible.

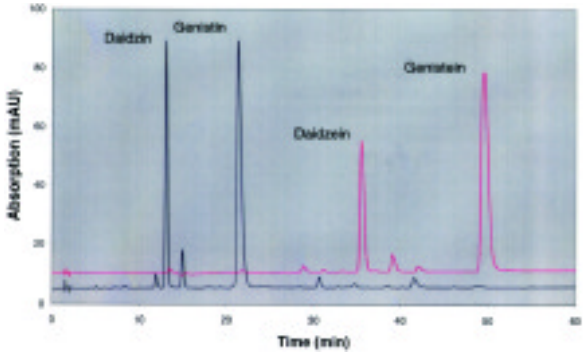


Fig. 4. HPLC analysis of isoflavones before (blue) and after (red) β-glucosidase treatment. Separation was done on a Merck Superspher 100 RP-18C column (4 µm, 254 x 4 mm) at 40°C. Solvant A was H2O + 0.1% acetic acid and solvant B acetonitril + 0.1% acetic acid. A gradient from 10% B to 30% B in 60 min was applied. Flow was 1.5 ml/min and detection was at 260 nm.

Application of soy isoflavone aglycones in cosmetic formulations

Over the last decade, plant derived ingredients have gained a lot of interest in cosmetic formulations. However, in many cases, these products do not offer the proposed activity since the preparations do not contain the active molecules in an appropriate concentration or the compounds are not bioavailable. The described liposomal aglycone preparation is a new active ingredient derived from soy. The preparation contains the active form of isoflavones in a high concentration and offers a number of different cosmetic and dermatological applications.

Oral and topical estrogen applications have shown to preserve collagen content, elastic properties, and thickness of the skin in postmenopausal women (*Dunn et al. 1997, Schmidt et al. 1996*). However, human hormones cannot be used in cosmetic formulations and due to the potential adverse effects should only be considered in severe cases in dermatological products. The treatment of skin during and after menopause with an active aglycone preparation from soy is a completly new approach in the battle against the aging process in the skin. The loss of hormonal activity reduces the skin tone and its hydration. This deterioration is based on the reduction of the metabolic activity of skin cells and results in wrinkles and dry skin.

Isoflavones aglycones encapsulated into liposomes can be applied in different formulations such as gels, lotions and creams since the product can easily be formulated into the water phase of cosmetics. A combination with moisturizers and classical anti-aging ingredients, such as vitamins and antioxidants may even further improve the activity of the product.

It is evident, that isoflavones are also very interesting compounds for anti-cellulite products as they enhance the skin tone and its viscoelastic properties. Thus, we use our aglycone preparation in the respective field. In our products, we combined the phytohormones with an algae extract (spirulina platensis) which is rich in minerals to achieve a highly efficient anti-cellulite formulation suitable to treat hormone dependent skin and subcutis problems (*Zülli et al. 2001*).

The anti-breast cancer properties of soy isoflavones have been reported (*Setchell and Cassidy 1999*). But only little data exist about the effects of soy isoflavones on histologically normal human breast. In one of these very intersting studies McMichael-Phillips et al. 1998 found that phytoestrogens can stimulate the proliferation of breast epithelium significantly in vivo based on higher serum levels of genistein and daidzein after soy supplementation. Thus the application of liposomal aglycones in breast firming products is a new opportunity to improve skin tone and elasticity in women of all ages.

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