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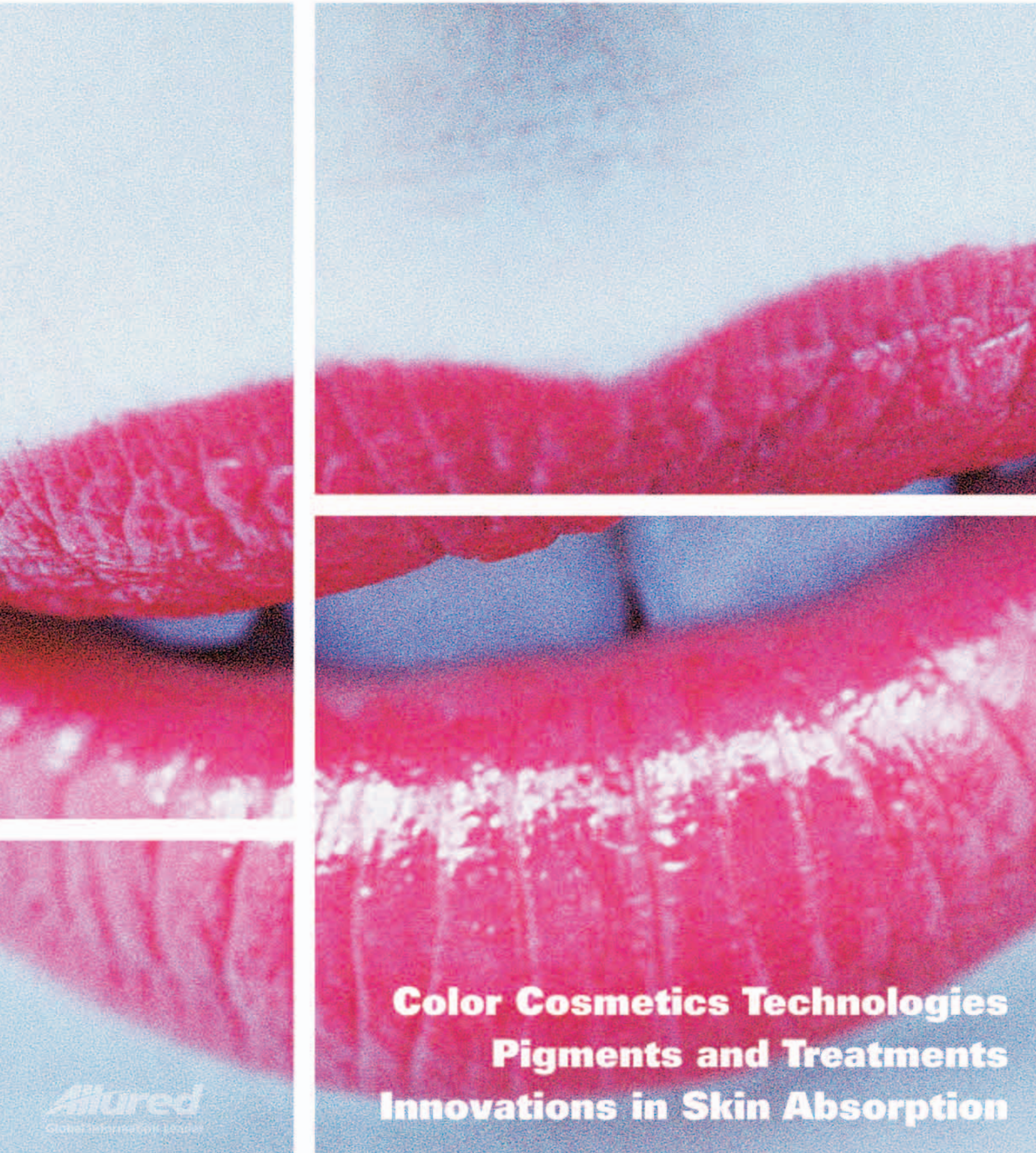

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Penetration and Metabolism of Isoflavones in Human Skin

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Key words

soy isoflavones, hydrolysis, liposomes, skin penetration, genistein

Abstract

This human study shows that the isoflavone aglycone genistein penetrates significantly better into the skin when it is formulated in a liposomal preparation. The corresponding isoflavone glycoside genistin is not hydrolyzed to the biologically active aglycones upon topical application.

Flavonoids form a large group of plant polyphenols, comprised of more than 5,000 molecules. The group is subdivided into anthocyanins, which are responsible for the colors from blue to red in flowers and fruits, and the colorless or white-to-yellow flavones, flavonols and isoflavones.

Many of these flavonoids are biologically active compounds that may have disease-preventing properties. Studies have been carried out mainly on cancer, cardiovascular disease and postmenopausal problems. In addition to their potent antioxidant properties, their mechanisms of action may include specific inhibition of enzymes or binding to receptors (Figure 1).

Most of the flavonoids in plants are found in the glycoside form, which means they are attached to a sugar residue.[1] As such, they are water-soluble and can be kept in the aqueous plant vacuoles. The glycosides are usually biologically inactive in humans because of low cellular uptake and because they do not fit into the binding sites of enzymes and receptors. After oral application, transformation into active molecules occurs through the action of hydrolytic enzymes in the intestine.[2]

Flavonoids as Cosmetic Ingredients

Flavonoids have become very popular as cosmetic ingredients, with claimed skin benefits such as prevention of lipid oxidation,[3] stimulation of fibroblast proliferation,[4] reduction of collagen breakdown,[5] and inhibition of 5α-reductase.[6] The prevalent isoflavone in soy is the glycoside genistin. Its aglycone (the molecular form without sugar residues), genistein, is a well-known inhibitor of protein tyrosine kinases.[7]

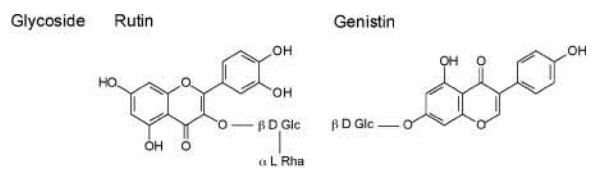

Figure 1. Glycoside structures, source materials and biological activities of the isoflavones quercetin and genistein

Kinases are involved in the signaling process inside cells. In the skin, they are essential components of the signal transduction pathway that is activated by radicals and inflammatory cytokines and leads to the expression of collagen- and elastin-degrading enzymes. These enzymes, called matrix metalloproteinases, are key players in skin aging.[8] Genistein can block this signaling pathway and as a consequence, radicals and inflammatory cytokines have no effect on collagen and elastin metabolism.

Most cosmetic ingredients based on isoflavones contain the glycoside forms. To become an active substance they must be hydrolyzed in situ after topical application.

In this work, we analyzed by tape stripping the penetration of isoflavones into the skin and the possible transformation of isoflavone glycosides to the corresponding biologically active aglycones.

Transformation of Prodrugs in Human Skin

The transformation of compounds in the skin was first raised when the concept of "prodrugs" was introduced into the cosmetic field. Prodrugs are

inactive compounds that are transformed into active molecules by enzymes at or near the site of action.

The concept was applied in the cosmetic field mainly for the highly unstable vitamins C and E. The water-soluble ascorbic acid (vitamin C) and the oil-soluble tocopherol (vitamin E) are the most prevalent antioxidants in the epidermis, and there is interest in delivering the antioxidant benefits of these vitamins to the skin. Unfortunately, these vitamins are very unstable in cosmetic preparations. Therefore, cosmetic formulations contain prodrugs in the form of stable esters of vitamins C and E, on the premise that enzymes in the epidermis will transform the provitamins into the active vitamins.

Although the stratum corneum contains very little free water, metabolic activity has been found in this layer of the skin. A tape-stripping technique has been used to measure the activities of enzymes that are important for maintaining the integrity of the stratum corneum. Enzyme activities have been shown for the phosphatases, phospholipases, and β-glucocerebrosidase that are involved in the formation of the multilamellar lipid layers, and for the serine-type proteases trypsin and chymotrypsin, which are responsible for the degradation of the corneodesmosomal linkages in the desquamation process.[9]

The data regarding bioconversion of vitamin C and E esters are conflicting. Regarding derivatives of vitamin C, Pinnell et al.[10] found no penetration of the water-soluble ascorbyl phosphate and no hydrolysis of ascorbyl palmitate. The latter seems to penetrate readily through the stratum corneum, but appears to remain on the extracellular surface of cells in the living epidermis. Thus, it seems that the ester derivatives are not taken up into the cells.[11]

Alberts et al.[12] showed absorption of α-tocopherol acetate into the skin but no evidence of its metabolism to the free form.

Rangarajan and Zatz[13] found that 15-20% of the α-tocopheryl acetate that permeated into the viable skin was metabolized into the active form. However, they could not detect any α-tocopherol in the stratum corneum, and the extent of permeation was shown to depend on the formulation.

Similar results were shown by Baschong et al.[14] They detected deposition in the underlying skin only when α-tocopheryl acetate was solubilized or encapsulated. Hydrolysis of α-toco-pheryl acetate did not occur on the skin surface or in the stratum corneum, but up to 50% of the compound was hydrolyzed in the layers below.

Hydrolysis of vitamin esters occurs only in the living part of the epidermis. Prior to conversion the esters therefore have to cross the stratum corneum. However, penetration seems to be critical and highly dependent on the formulation. The rate of hydrolysis in the deeper epidermis is still a matter of discussion.

Materials and Methods

Three different isoflavone preparations (Formula 1) were applied (50 µl/cm²) to test areas on the inside of the forearm.

After 4 hours, skin samples were taken by stripping the test areas with adhesive tapes (width: 2 cm; length: 2.5 cm) to analyze the distribution profile of the ingredient (the dose of genistein applied on the stripped area was 750 µg). This method gently removes the stratum corneum. After solubilization of the material from the tapes, the samples were filtered and concentrated on a rotary evaporator.

The concentration of genistein was analyzed by HPLC: the dried extracts were dissolved in 250–µl acetonitrile and applied to an RP-18 column[a]. The mobile phases were water with 0.1% acetic acid (A) and acetonitrile with 0.1% acetic acid (B). The gradient was 10% B to 30% B over 60 min. Detection was carried out at 260 nm.

Results and Discussion

In order to analyze penetration of the isoflavone genistein and to verify whether its glycoside is hydrolyzed in skin, we conducted a study with ten human volunteers and three different test preparations:

- Formula 1a – Isoflavone aglycone genistein with liposomes;
- Formula 1b – Same as Formula 1a but without liposomes to address their influence on penetration;
- Formula 1c – Isoflavone glycoside genistin in liposomes to determine the rate of hydrolysis.

The method of tape stripping was used to analyze the distribution of genistein in the skin after application of the different preparations. The study results are shown in Figure 2.

The genistein in Formula 1b remained mainly in the outermost stratum corneum (strips 1 to 5). Only 4% of it was found in strips 6 to 10 and nothing in deeper layers. The distribution profile of the genistein in Formula 1a compared to that of the genistein in Formula 1b was clearly shifted to deeper stratum corneum layers. The largest portion of genistein was detected in strips 6 to 10. In the skin areas where Formula 1c was applied, no significant amounts of genistein could be detected in the stratum corneum.

The recovery yield of the detected genistein in the HPLC samples was 54%, 42% and 0.3% for Formulas 1a, 1b and 1c compared to applied amounts of genistein or genistin, respectively.

Conclusions

Our study clearly shows that isoflavone glycosides are not hydrolyzed in significant amounts four hours after application. The results indicate that the reported minimal enzymatic activity in the stratum corneum[9] is apparently not enough to significantly hydrolyze glycosides. Therefore, topically applied isoflavone glycosides cannot exert significant physiological effects in the skin.

The tape stripping results show that liposomes can tremendously enhance the penetration of isoflavones. Instead of remaining in the outermost layers, isoflavones in liposomes penetrate into deeper layers of the stratum corneum (strips 2 to 10). But there is no significant penetration of genistein through the stratum corneum into the fully metabolically active epidermis after a single application.

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Formula 1. Isoflavone test preparations

	a	b	c
Genistein	0.3%	0.3%	-
Genistin*	-	-	0.55%
Phospholipids	5.0	-	5.0
Polysorbate 80	10.0	10.0	10.0
Ethanol	21.0	21.0	21.0
Water (*aqua*)	ad 100.0	ad 100.0	ad 100.0

a) aglycone genistein with liposomes
b) aglycone genistein without liposomes
c) glycoside genistin in liposome

* Same molar concentration as in the genistein preparations

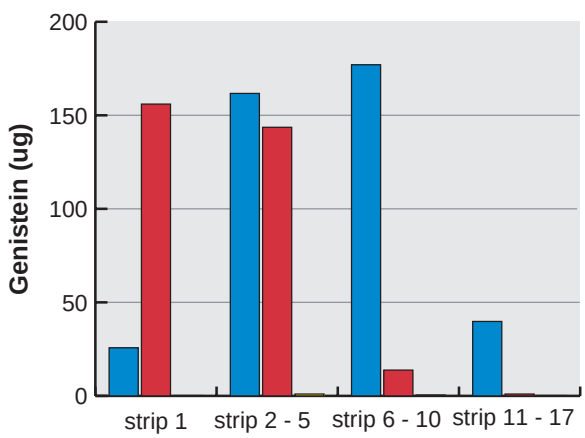

Figure 2. Percutaneous absorption and metabolism of soy isoflavones, indicated by genistein found on stripped stratum corneum after application of the following test preparations: 0.3% genistein in liposomes (blue); 0.3% genistein without liposomes (red); and 0.55% genistin in liposomes (yellow).

[a] *LiChroCART 250-4 Superspher 100 Merck, Germany*

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