

How to obtain skin whitening in a safe and effective way?

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Abstract

Melanin the natural color in our skin is synthesized by melanocytes. The distribution pattern in the surrounding keratinocytes and the nature of the formed melanin determine the actual color of our skin. Melanin forms through a series of oxidative reactions involving the amino acid tyrosine in the presence of the enzyme tyrosinase. Skin whitening agents often inhibit the activity of tyrosinase, for example, by competitive or non-competitive inhibition of its catalytic activity, by inhibiting its maturation, or by accelerating its degradation. The cosmetic industry is looking for novel, effective, possibly "natural" ingredients of low or absent side effects. With hydroquinone, the previous benchmark in this field, fast and effective results to whiten skin were obtained. The drawback of the fast effect was the high cytotoxicity of hydroquinone and its huge irritation potential. New active ingredients with a much improved toxicology profile have been developed instead. They often provide the whitening effect in a reversible way, demonstrating that the biology of melanin production will only be suppressed during treatment but not destroyed. These new kinds of natural whitening ingredients however force us to become more patient. They do work well if they get the time the biochemistry of the skin needs and surprisingly they can even provide skin protection.

Introduction

Up to 10% of skin cells in the basal layer of the epidermis produce melanin. Upon exposure of the skin to UV irradiation, melanogenesis is initiated with the first step of tyrosine oxidation through tyrosinase. Tyrosinase is a multifunctional, glycosylated, copper containing oxidase. It is synthesized by melanosomal ribosomes found on the rough endoplasmic reticulum (ER). After synthesis, tyrosinase is glycosylated. It is subsequently delivered to melanosomes via coated vesicles in an inactive form. The biosynthetic pathway for melanin formation in various life forms has largely been elucidated by Raper (1928), Mason (1948) and Lerner *et al.* (1949).

Melanins also play a crucial role in the absorption of free radicals generated within the cytoplasm and in shielding the host from various types of ionizing radiations, including UV light. Melanins can be of two basic types: eumelanins, which are brown or black, and pheomelanins, which are red or yellow. The metabolic pathways are illustrated in Figure 1. In mammals, mixtures of both types are typically found [1].

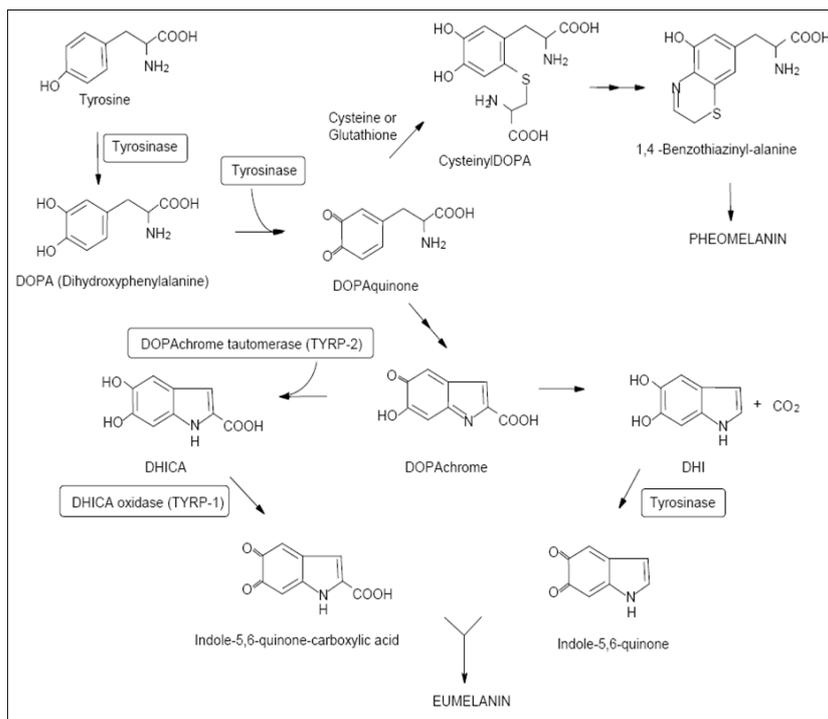


Figure 1 Biosynthetic pathway of melanin formation

Multiple potential approaches exist that could control pigmentation via the regulation of tyrosinase activity. The transcription of its messenger RNA, its maturation via glycosylation, its trafficking to melanosomes, as well as modulation of its catalytic activity and/or stability could be regulated. Tyrosinase is produced only by melanocytic cells and following its synthesis and subsequent processing in the endoplasmic reticulum (ER) and Golgi apparatus, it is trafficked to specialized organelles, termed melanosomes, wherein the pigment is synthesized and deposited [2].

Well known tyrosinase inhibitors

1. Kojic Acid

Kojic acid (Figure 2) is derived from Koji (a Japanese mushroom). Kojic acid lightens the skin by inhibiting tyrosinase which in turn reduces the amount of melanin produced. Kojic acid is one of the most popular natural ingredients found in skin lightening products. However, Kojic acid has the potential to induce skin sensitization according to the Scientific Committee on Consumer Products (SCCP) of the EU [3].

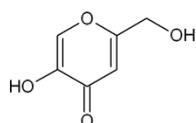


Figure 2 Chemical structure of Kojic acid

2. Mulberry

This skin lightening ingredient is extracted from the roots of the paper mulberry plant. Mulberry is said to be more effective than hydroquinone and kojic acid in the sense that significantly lower concentrations are needed to have the same effect as higher concentrations of kojic acid and hydroquinone.

3. Alpha Arbutin

Alpha arbutin (Figure 3) has a stronger effect than beta-arbutin and is also commonly found in skin lightening products as a safer alternative to hydroquinone.

4. Beta Arbutin (Bearberry Extract)

Beta arbutin (commonly known as just arbutin, Figure 3) is derived from the leaves of bearberry, cranberry and blueberry plants. It works in a similar way to kojic acid, in

that it inhibits the production of tyrosinase to restrict the amount of pigment produced. Although naturally derived, it can cause skin irritation in some people with sensitive skin.

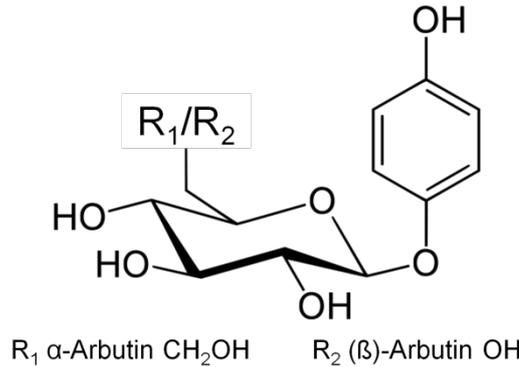


Figure 3 Chemical structure of α -Arbutin and (β)-Arbutin

5. Glutathione

Glutathione is a powerful antioxidant that has several health benefits including boosting the immune system and cleansing the liver. A side effect of glutathione is the direction of melanin production towards the light and easy soluble pheomelanin thus supporting skin whitening [4]. Glutathione is commonly found in skin lightening pills but can also be used topically. It is naturally occurring in our body and is a part of our natural defense system.

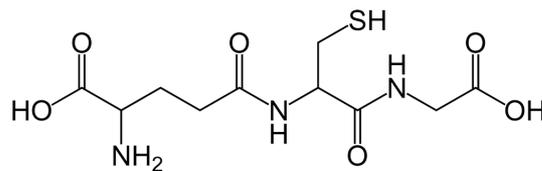


Figure 4 Glutathione skeletal

6. Licorice Root

Licorice is commonly used in the skin lightening industry. Licorice also works to inhibit the enzyme tyrosinase to limit the amount of pigment produced. It has additionally anti-inflammatory properties and is particularly effective at fading suntans.

7. Papaya

The papain enzyme found in papaya works to gently exfoliate dead skin cells and reveal new, brighter skin cells beneath. Orange and green papaya are both effective but green papaya contains more of the papain enzyme. This ingredient is most often found in soaps but sometimes in skin lightening creams too.

8. Vitamin A (Retinol)

Vitamin A, also known as retinol (Figure 5) increases the rate at which skin cells are renewed. Increased skin cell turnover means that the brighter, fresh skin underneath can be revealed.

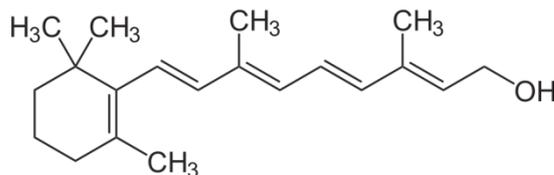


Figure 5 All-trans retinol

9. Vitamin B3 (Niacinamide)

Niacinamide (Figure 6), also known as nicotinamide or Vitamin B3 has antioxidant and anti-inflammatory properties. Niacinamide is effective topical skin lightener that

works by disrupting the transfer of melanosome from the melanocyte to the keratinocyte. Vitamin B3 is found in some skin whitening products and sun screens.

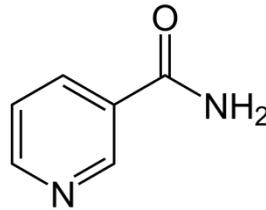


Figure 6 Niacinamide

10. Vitamin C

Vitamin C suppresses the production of pigment in the skin. Magnesium Ascorbyl Phosphate is a derivative of Vitamin C and this is the form of the ingredient that is commonly found in skin whitening products. Vitamin C can also protect the skin from ultraviolet rays.

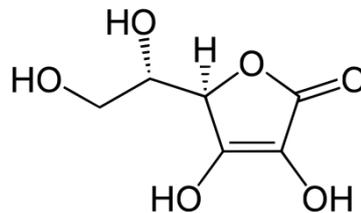


Figure 7 L-Ascorbic acid

All these ingredients are not usually intended to work in isolation. An effective skin lightening treatment combines several of these ingredients as they tend to work well together to produce the desired results.

Mechanisms to inhibit melanogenesis

Inhibition of tyrosinase mRNA transcription

Melanin synthesis is directly regulated by the enzymatic function of tyrosinase and thus by transcription of its encoding gene. Decreases of tyrosinase mRNA levels in cultured melanoma cells can be elicited by incubation with a number of substances like e.g.

- Transforming growth factor- β 1 (TGF- β 1) (Martinez-Esparza et al., 1997)[2]
- Tumor necrosis factor- α (TNF- α) (Martinez-Esparza et al., 1998)[2]

Microphthalmia associated transcription factor plays a role in the development, survival, and function of certain cell types. To carry out this role, the protein attaches to specific areas of DNA and helps control the activity of particular genes. Microphthalmia-associated transcription factor helps control the development and function of melanocytes. Within these cells, this protein also controls production of the pigment melanin, which contributes to hair, eye, and skin color. This protein is the master regulator of melanogenesis-related gene expression (Tachibana et al., 1996). A few examples of factors that decrease levels of mRNAs encoding tyrosinase and/or microphthalmia associated transcription factor in cultured melanoma cells, melanocytes or melanoblasts are [2,5]:

- Agouti signal protein (Aberdam et al., 1998)
- Hydrogen peroxide (Jimenez-Cervantes et al., 2001)
- Sphingosine-1-phosphate (Kim et al., 2003)
- (-)-Epigallocatechin-3-gallate and hinokitiol (Kim et al., 2004)

Aberrant tyrosinase maturation

Tyrosinase is a glycoprotein with six N-linked glycosylation sites that are conserved in human and mouse tyrosinases (Kwon et al., 1987; Müller et al., 1988; Ujvari et al., 2001). Aberration of tyrosinase glycosylation in the ER or Golgi inhibits its folding and maturation and results in hypopigmentation. Some factors have been shown to inhibit melanogenesis in cultured melanoma cells or in melanocytes by modulating the glycosylation of tyrosinase, for example [2]:

- Glutathione (Imokawa, 1989)
- Ferritin (Maresca et al., 2006)
- Feldamycin (Terao et al., 1992)
- Calcium D-pantetheine-S-sulfonate (Franchi et al., 2000)

Inhibition of tyrosinase catalytic activity

Numerous reports have described the inhibition of tyrosinase activity, many of them using mushroom tyrosinase as a model. However, the regulation of mushroom tyrosinase differs significantly in several respects from mammalian tyrosinase (Pomerantz, 1963; Hearing et al., 1980). Inhibitors of tyrosinase function can be divided into two groups, competitive inhibitors and non-competitive inhibitors. Early studies revealed that hydroquinone and azelaic acid, both being potent therapies for hyperpigmentary disorders (Arndt and Fitzpatrick, 1965; Grimes, 1995), are competitive inhibitors of tyrosinase activity as well having cytotoxic effects on melanocytes (Jimbow et al., 1974; Nazzaro-Porro and Passi, 1978; Nazzaro-Porro et al., 1979; Palumbo et al., 1991). Arbutin, a b-D-glucopyranoside derivative of hydroquinone, also inhibits tyrosinase activity competitively but at non-cytotoxic concentrations in cultured melanocytes (Maeda and Fukuda, 1996). Competitive inhibition can also result from treatment of cultured melanocytes, melanoma cells or purified tyrosinase with 4-tertiary butylphenol (Yang and Boissy, 1999), aloesin (Jones et al., 2002), 4,40-dihydroxybiphenyl (Kim et al., 2005d), and 4-n-butylresorcinol (Kim et al., 2005a).

Tyrosinase activity depends on the binding and function of two copper atoms at the active site (Furumura et al., 1998; Branza-Nichita et al., 1999) which is facilitated by a copper transporter (Petris et al., 2000). Therefore, chelating copper inhibits the activity of purified or recombinant tyrosinase, for example, by phenylthiourea (PTU) (Dubois and Erway, 1946), by kojic acid (Mishima et al., 1988) or by ellagic acid (Shimogaki et al., 2000).

Another non-competitive method of inhibiting tyrosinase activity is the reduction of its phosphorylation. Therefore, inhibiting tyrosinase phosphorylation reduces tyrosinase activity in cultured melanocytes as well as lightening pigmentation in the skin and hair. Further, since the second reaction in the melanogenic cascade as well as other non-enzymatic reactions in the pathway, are oxidative reactions, antioxidants such as a-tocopheryl ferulate, magnesium L-ascorbyl-2-phosphate and 2-O-a-D-glucopyranosyl-L-ascorbic acid, are also effective inhibitors of melanin synthesis in cultured melanoma cells. [2]

Acceleration of tyrosinase degradation

The synthesis and the degradation of tyrosinase are tightly coupled to its function, and are influential parameters that regulate melanin synthesis. When a melanogenic inhibitor decreases tyrosinase protein levels but has little effect on its mRNA levels, it is like that the degradation of tyrosinase was accelerated by that agent. The degradation of tyrosinase could be altered by environmental factors surrounding melanocytic cells. Studies of tyrosinase degradation have revealed that a variety of intrinsic factors in the epidermis or other factors have a potency to regulate tyrosinase degradation. Keratinocytes synthesize and secrete various cytokines such as IL-1a and TNF-a, IL-6, TGF-b1 and pro-opiomelano-cortin (POMC, the precursor for melanocyte stimulating hormone). Among those cytokines, TGF-b1 enhanced in a dose-dependent manner the inhibition of tyrosinase and TYRP1 activities in B16 mouse melanoma cells following treatment with cycloheximide. [2]

Linoleic acid is an unsaturated fatty acid (C18:2) and is a major component of biological cell membranes. Topical application of linoleic acid has been shown to decrease UV-induced hyperpigmentation of the skin. Fatty acids can regulate tyrosinase degradation in contrasting manners. Linoleic acid accelerates whereas palmitic acid (saturated fatty acid, C16:0) decelerates the degradation of tyrosinase, with little change in levels of tyrosinase mRNA.

[2,6]

A biphenyl derivative, 2,20-Dihydroxy-5,50-dipropyl-biphenyl (DDB) has a phenol structure that could potentially elicit competitive inhibition of tyrosinase as does hydroquinone and

arbutin, although it does not directly affect levels of tyrosinase protein or mRNA. DDB downregulates melanin synthesis by inhibiting the maturation of tyrosinase leading to an acceleration of tyrosinase degradation. DDB also inhibited the trafficking of tyrosinase to melanosomes due to the suppression of tyrosinase maturation. [2, 6]

Indirect regulation of tyrosinase activity

Epidermal pigmentation involves not only the intracellular regulation of tyrosinase activity within melanocytes but also reflects environmental influences on melanocytes, including those derived from surrounding keratinocytes. Many studies have attempted to inhibit melanin synthesis and/or tyrosinase activity indirectly by interfering with cutaneous secretory factors that can activate melanocytes. Examples include the inhibition of cell-to-cell signaling between keratinocytes and melanocytes which activates melanogenesis via paracrine cytokines, by inhibitors of inflammation such as by glabridin found in licorice extracts (Yokota et al., 1998). [2]

New kinds of natural whitening ingredients

In the large variety of nowadays commercially available whitening products, the use of different natural whitening agents is well noticeable.

The utilization of kojic acid and arbutin is still common because these agents have repeatedly been demonstrated to be effective whitening agents. The use of bearberry extracts (a natural source of β -arbutin) may strengthen the effect of α -arbutin. Among the natural extracts, mulberry and licorice are popular components added to the skin whiteners. The isolation of their active components and their effect on tyrosinase inhibition and pigment reduction has already been described.

Also lemon extract is used as a potent skin bleaching ingredient. However, it can only be used at low concentrations because it easily causes skin irritation.

Several studies describe *Sophora* species from which several active compounds have been isolated that act as potent inhibitors of tyrosinase and pigment production [7].

Niacinamide, which besides inhibition of tyrosinase, interferes in melanosome transfer to keratinocytes is also used and was described already earlier. The Mandresy extract contains two compounds luteolin and verbascoside that do not only inhibit tyrosinase and pigment production but also influence the interaction between keratinocytes and melanocytes by reducing formation of dendrites.

Some whitening products contain a mixture of many extracts with the obvious tyrosinase inhibitors (Mulberry, Licorice, Sophora and Peonia) but also other extracts that may act as antioxidant or anti-inflammatory agent additionally. Another component contains various plant extracts from the Alps tested on subjects of Asian origin. Its bleaching effects may partly be attributed to tyrosinase inhibition.

The question arises whether the increasing amounts of potentially active whitening ingredients will cause additive effects or will reduce the effects of the most potent ingredients.

Some companies still use single synthetic compounds, for instance dimethylmethoxy chromanyl palmitate which exhibits lightening activity in Asian volunteers after 30 and 60 days. A new mechanism of action is targeting the peroxisome proliferator-activated receptor (PPAR). The active ingredient is able to reduce tyrosinase mRNA expression [8].

Thus, approaches for skin whitening have broadened widely in the recent years. The utilization of single agents inhibiting tyrosinase is in many cases extended to the use of complex mixtures that target different mechanisms like tyrosinase expression, transfer of melanosomes, antioxidant and anti-inflammatory effects.

Many different plant names are mentioned in this article and one can find even more within the references. There is one which hasn't been mentioned and is so well known for many years, not just in the western hemisphere. It is *Olea europaea*. While mainly the virgin oil is used as an emollient with excellent skin properties, the more water soluble parts of *Olea europaea* fruits and leaves provide strong anti-oxidative and radical-scavenging properties.

Standardised extracts with lead molecules like hydroxytyrosol which is formed of Oleuropein during maturation and a number of other olive polyphenols like tyrosol, gallic acid, ferulic acid and caffeic acid [9] provide skin whitening and age spot reduction.

The effect is based on two mechanisms:

1. The extract directly inhibits melanin formation in vitro on human melanocytes (Figure 8) by inhibiting tyrosinase maturation and possibly other effects. The reason might be an increase of the glutathione level (Figure 9). The complex effects of *Olea europaea* on skin whitening could not be sufficiently explained yet.
2. The increased glutathione level provides two major effects.
 - a. It leads to the formation of pheomelanin, the light and easy soluble melanin type (Figure 1).
 - b. At the same time glutathione acts as the strong body owned antioxidant and protects the skin against oxidative stress whether initiated by UV-rays or autoxidation or other sources (Figure 10).

Publications show that hydroxytyrosol also protects human melanocytes in vitro from protein damage, induced by long-wave UV light, and reduces the release of inflammation inhibitors like Cox-2 in macrophages [10,11].

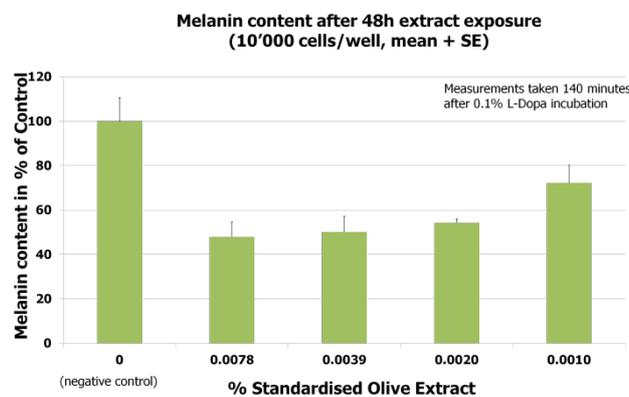


Figure 8 Reduction of melanin in vitro on human melanocytes

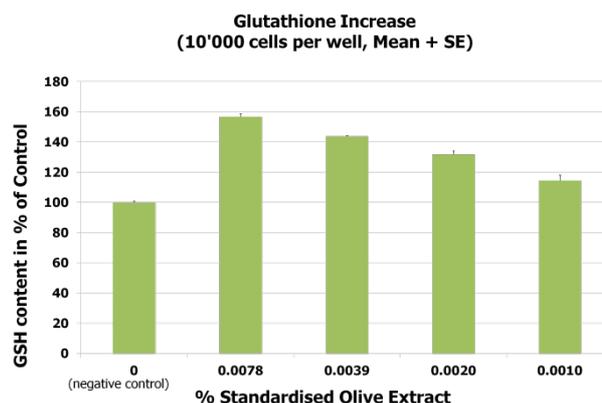


Figure 9 Glutathione increase in vitro on human melanocytes

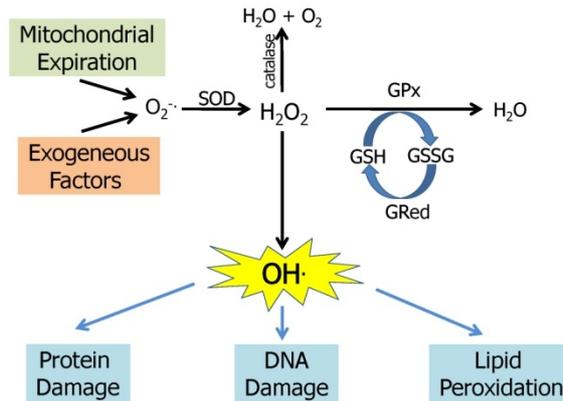


Figure 10 Task of Glutathione

A standardized olive extract with high concentration levels of the lead substances can be used at very low levels in cosmetic formulations. An example shows that a hand cream with only 0.2% concentration level and no other whitening active showed significant reductions in skin color as well as age spots (Figure 11). The only drawback was that the biochemistry in skin needed its natural renewal time of the epidermis to work successfully. Since the average age of the volunteers was above 50 years a renewal time of 6 weeks was estimated which let the test last for 3 months to pass two renewal cycles. Interestingly the whitening effect is reversible after treatment stop which indicates that the natural pigmentation process of skin was just suppressed, however not destroyed by the natural treatment.

Female Volunteer Caucasian, 49 years old with very dry skin and Sun Sensitivity (Fitzpatrick) of 2



Placebo Before the Treatment, D0 Treatment

Figure 11a Female volunteer before treatment

Female Volunteer Caucasian, 49 years old with very dry skin and Sun Sensitivity (Fitzpatrick) of 2



Placebo After the Treatment, D84 Treatment

Figure 11b Female volunteer after treatment

Conclusion

Skin whitening has been extensively examined during the last decades. A number of different pathways were evaluated to achieve a reduction in skin color formation. With the different pathways whitening agents were developed and entered the market quickly specifically where a pale skin complexion is preferred.

The first whitening ingredients should just perform, no matter whether there could side effects been expected or not. With time and a better communication regime like e.g. the internet consumers became much better informed and aware of potential risks. They finally wanted safer skin whitening products with less side effects.

Many different skin whitening agents have been mentioned in this article. Obviously they are all less dangerous than the first benchmark hydroquinone, however, not all of them are totally safe either and should be used carefully.

During the last years with a strong booming green chemistry new ingredient idea were developed taking the know-how of mother-nature into account. The last example of a natural ingredient mixture from a well known plant which has been used for many centuries demonstrates that skin whitening nowadays can be achieved by safe ingredients and instead of toxicological drawbacks with an extra benefit namely additional skin protection.

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