KaliLight

FUNCTIONALIZED HYDROXYAPATITE

Innovative skin lightening
active ingredient

Increases the cutaneous
brightness

Effective against

Hyper pigmentation
and skin spots

Kalichem Italia s.r.l.

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A white, micro-fine, stable powder of activated hydroxyapatite particles is used as a biocompatible delivery system to enhance the synergy of Cysteine and Glutathione, as skin lightening and de-pigmenting complex.

>> INTRODUCTION

Hydroxyapatites are naturally occurring mineral forms of calcium apatite, with the general formula $Ca_5(PO_4)_3(OH)$. Hydroxyapatite is the hydroxyl end-member of the complex apatite group, where the OH- ion can be replaced by fluoride, chloride or carbonate ions. It crystallizes in the hexagonal crystal system, has a specific gravity of 3.08 and occupies the 5th position in the Mohs hardness scale. Pure hydroxyapatite powder is white. However, naturally occurring apatites can also be brown, yellow or green. 70% of the human bone is made of the inorganic mineral hydroxyl-apatite (1). Carbonated-calcium deficient hydroxyl-apatite is the main mineral of which dental enamel and dentin are composed of.
Hydroxyl-apatite can be found in teeth and bones in the human body. Scientific studies report its medical use as a filler in amputated bones or as a coating to promote bone ingrowth into prosthetic implants. It has been suggested that this material may promote ossification (2, 3, 4) and many modern implants are coated with hydroxyl-apatite. Recently, hydroxyl-apatite has been used as a semi-permanent filler in non-surgical options for treating wrinkles and textural changes in skin rejuvenation. Moreover, as the main component of dental enamel, it is reported to protect it from acid erosion (5, 6, 7) and to exhibit enamel restoring effects, as well as an anti-plaque and anti-stain activity.

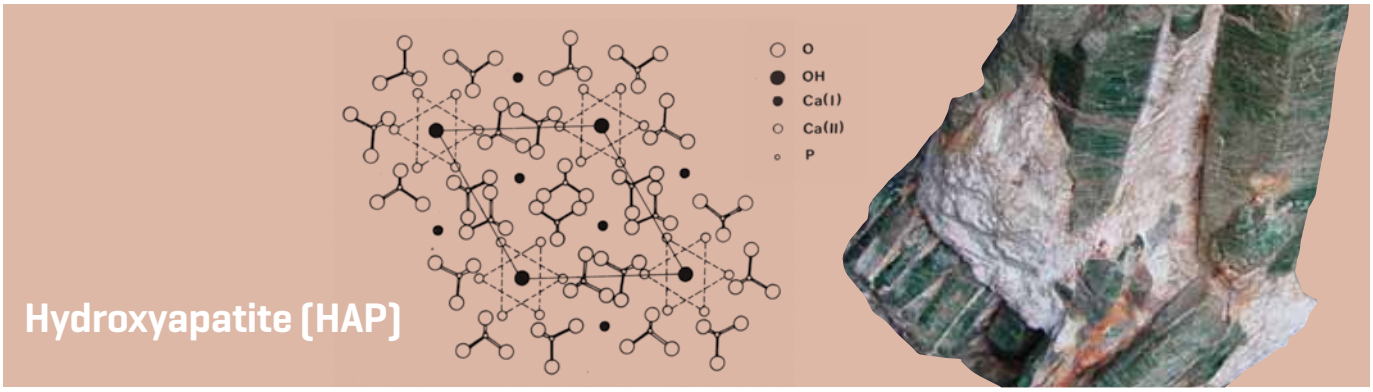


Figure 1: Projection of the constituting ions of hydroxyapatite on the basal (001) plane

>> FUNCTIONALIZATION

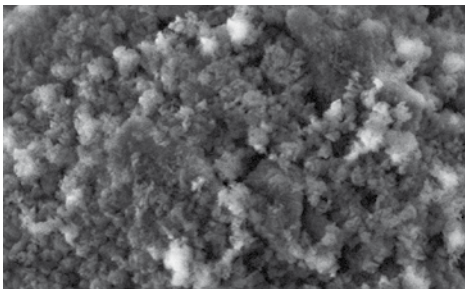


Figure 2: An image of HAP in a scanning electron

It is possible to calibrate the physical and chemical properties of synthetic HAP for specific biomedical applications. By developing new routes or modifications of pre-existing methods to produce HAP crystallites with reduced particle size and increased surface area of controlled morphology, or both. In particular, in order to have these biomedical applications, HAP must be produced in small size, similar to the biogenic one, it must have the same morphology, not too crystalline, the presence of carbonate ions in the crystal lattice, a comb-like morphology, and, finally, must have a fixed ratio Ca / P. As far as the cosmetic field is concerned, its characteristic as a slow-release source of Phosphate and Calcium ions to the skin cells suggests its use as an anti-aging ingredient for aged skin. HAP is 'highly hydrophilic, but insoluble in water at pH > 5. The particles have a very great surface, full of irregular protrusions and cavities. Moreover, it has been possible to extend and fine-tune the bioactivity of this compound by functionalization of water-soluble biomolecules. In this regard, amino acids are ideal candidates for the production of bio-inorganic HAP due to their intrinsic biocompatibility and ability of interacting with HAP surfaces (8). The affinity between protein and inorganic substrates can be due to different types of interactions: covalent and electrostatic bonds, Van Der Waals interactions. The relative predominance of one of these is closely related to protein structure and the chemical and physical characteristics of the inorganic surface. In the case of biomimetic HAP the adsorption or the release of protein and amino acids is influenced by size variation, morphology, surface area, Ca / P surface ratio. Therefore, the system can be used as a biocompatible delivery system able to regulate the release of actives into the skin.

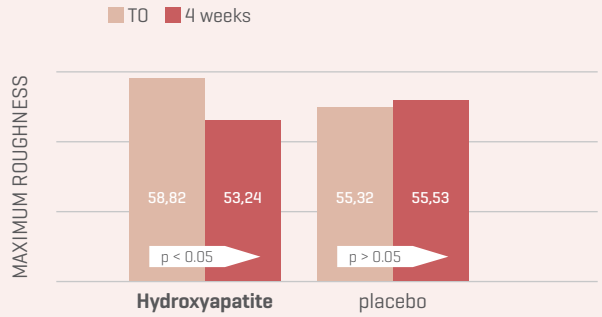
Kalilight is a functionalized hydroxyapatite able to effectively deliver Cysteine and Glutathione and enhance their synergic activity and efficacy as skin lightening and de-pigmenting complex.

>> ROUGHNESS, ELASTICITY, ANTI-AGE AND SOFT FOCUS EFFECT OF HYDROXYAPATITE

Remarkable results were found, instead, in the case of skin roughness, where there is a variation of -9,5% (p<0,05). Also the skin elasticity results were significant, especially the "Biologic elasticity": +10,3%, p<0,05 and the "visco-elasticity coefficient": -17,1%, p<0,01. Interesting results show where the soft-focus effect of Hydroxyapatite is evident. Particularly, 6 volunteers out of 10 showed a decrease in the fine lines, while 3 volunteers out of 10 showed a decrease in the deep wrinkles volume.

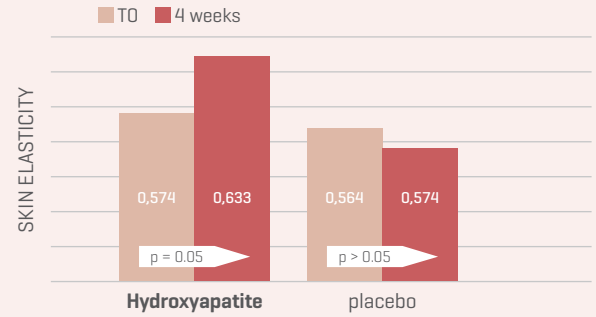
SKIN ROUGHNESS

-9.5% (p<0.05)



SKIN ELASTICITY

+10.3% (p<0.05)



SOFT-FOCUS EFFECT

Hyperpigmentation and Melanin Pathway

In many regions of the world, having a light and even skin colour is highly valued. There is a big number (approximately 60-75%) of women that desire to achieve a lighter skin colour. In addition, many hyperpigmentary skin disorders such as melasma, postinflammatory hyperpigmentation and senile lentigines can be exacerbated by sun exposure. One effective way to maintain a light and even skin colour and to ameliorate hyperpigmentation is to avoid sun exposure by using sunscreens or by wearing protective clothing. Jonker et al. [[i]] proposed that the regular use of sun protection products may lead to skin lightening, as the primary stimulus for facultative melanin synthesis is excluded and pigment that is present in the epidermis is lost through stratum corneum desquamation. The biosynthetic pathway for melanin formation in various life forms has firstly been elucidated by Raper (9), Mason (10) and recently has been modified by Cooksey et al. (11) and Schallreuter et al. (12) (Figure 3). Melanogenesis is initiated at first by tyrosine oxidation to dopaquinone catalyzed by tyrosinase. This is the rate-limiting step in melanin synthesis because the remainder of the reaction sequence can proceed spontaneously at physiological pH value (13). The resulting dopaquinone is converted to dopa and dopachrome through auto-oxidation. Finally, eumelanin is formed through a series of oxidation reactions from dihydroxyindole (DHI) and dihydroxyindole-2-carboxylic acid (DHICA), which are the reaction products of dopachrome oxidation (14).

>> SKIN LIGHTENING AGENTS

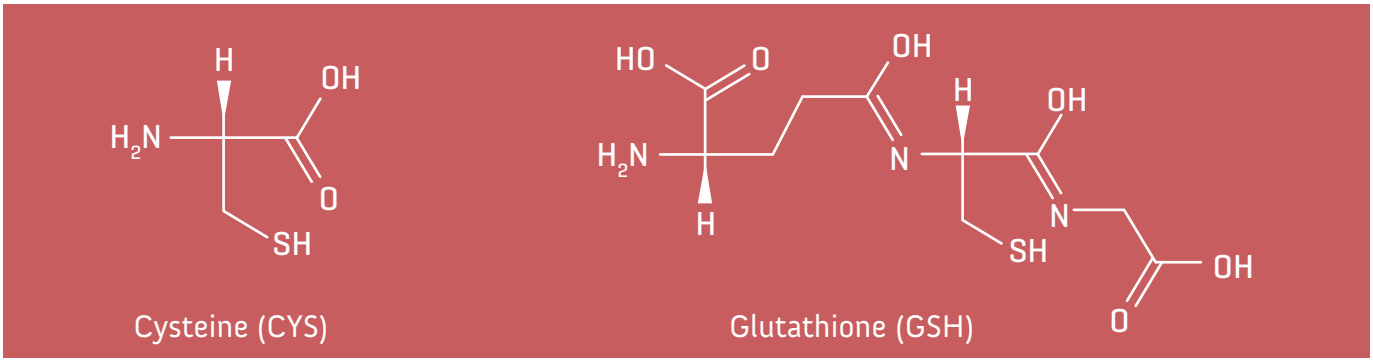
Numerous types of skin care products containing purported skin lightening agents such as arbutin and kojic acid, are commercially available. These skin lightening products suppress or inhibit melanogenesis in various ways [[ii]].
The problem linked to this ingredients is the toxicological profile. Hydroquinone has been used since the 1950s in commercially available over the-counter skin lightening products and since the 1960s as a commercially available medical product. It is also used in cosmetic products such as hair dyes and products for coating finger nails. Beginning in 2001, HQ is no longer authorized for use in cosmetic skin lightening formulations in European Union countries. However many products containing arbutin, an analogue of HQ, continue to remain available in European countries [[iii]].

Arbutin is reported to be converted to HQ. Among a group of human volunteers who ingested arbutin (equivalent to 168 mg of HQ), 70.6% of the dose of arbutin was excreted in the urine as HQ or its conjugates. A recent study has preliminarily reported the detection on the skin of HQ after application of both a-and-b-arbutin [[iv],[v]].

Kojic acid, a secondary metabolic product of various species of Aspergillus and Penicillium, has found widespread use as a food additive for preventing enzymatic browning of raw crabs and shrimps and as cosmetic agent for the purpose of skin lightening. These uses are based on its excellent inhibitory action on the polyphenol oxidase (tyrosinase) of crustaceans and on human melanocytes. However since 1968 several studies have clearly confirmed, besides its allergenic potential, the carcinogenicity of Kojic Acid in mouse liver [[vi]].

Cysteine and Glutathione

Cysteine (CYS) and Glutathione (GSH) are, respectively, an-amino acid and an ubiquitous tripeptide (cysteine-glycine-glutamate) found in our bodies.



Cysteine (CYS) Glutathione (GSH)

Aside from their many ascribed biological functions as antioxidants, they have also been employed in skin lightening. Apparently, the main reported mechanisms of action for glutathione include: (a) direct inactivation of the enzyme tyrosinase by binding with the copper-containing active site of the enzyme; (b) mediation of the switch mechanism from eumelanin to phaeomelanin production; (c) quenching of free radicals and peroxides that contribute to tyrosinase activation and melanin formation; and d) modulation of depigmenting abilities of melanocytotoxic agents.
Moreover, several experiments show that the synergic addition of glutathione with another thiol, cysteine, caused inactivation of melanoma tyrosinase in a dose-dependent manner (15, 16). In particular Cysteine quickly binds with dopaquinone through a non-enzymatic reaction to give rise to cysteinyldopas. These molecules undergo oxidative cyclization of their cysteinyl residues and are then converted to benzothiazine metabolites and ultimately to phaeomelanins or mixed type melanins called trichromes (17).

The hypothesized mechanism is well illustrated in Figure 3.

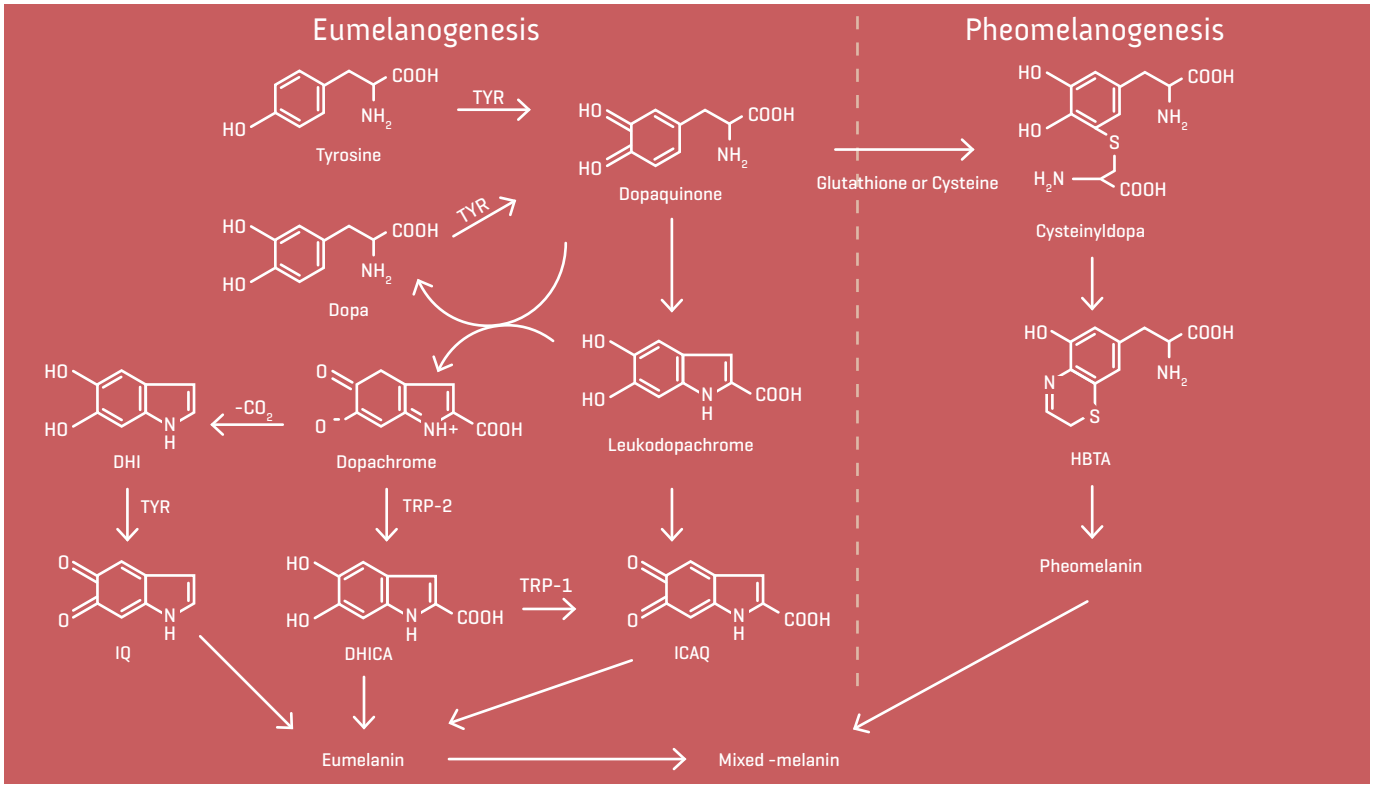


Figure 3: Biosynthetic pathway of melanin (14)

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Considering the above elements and scientific literature, the use of this new complex allows to associate the characteristics of hydroxyapatite with glutathione and cysteine in order to achieve a multi-function effect and, above all, a highly biocompatible ingredient. Indeed, all these ingredients Hydroxyapatite, Glutathione and Cysteine are produced in the human organism with different physiological functions. The first effect, related to Hydroxyapatite, is the slow release of phosphate, calcium and other ions at the skin slightly acidic pH when the ingredient comes into contact with the skin. These ions improve skin health for their lenitive and nourishing effects.
The second effect, related to the previous one, is the consequent slow release of the skin lightening complex made of glutathione and cysteine. This results in a more physiological and biocompatible skin intake of the ingredients.

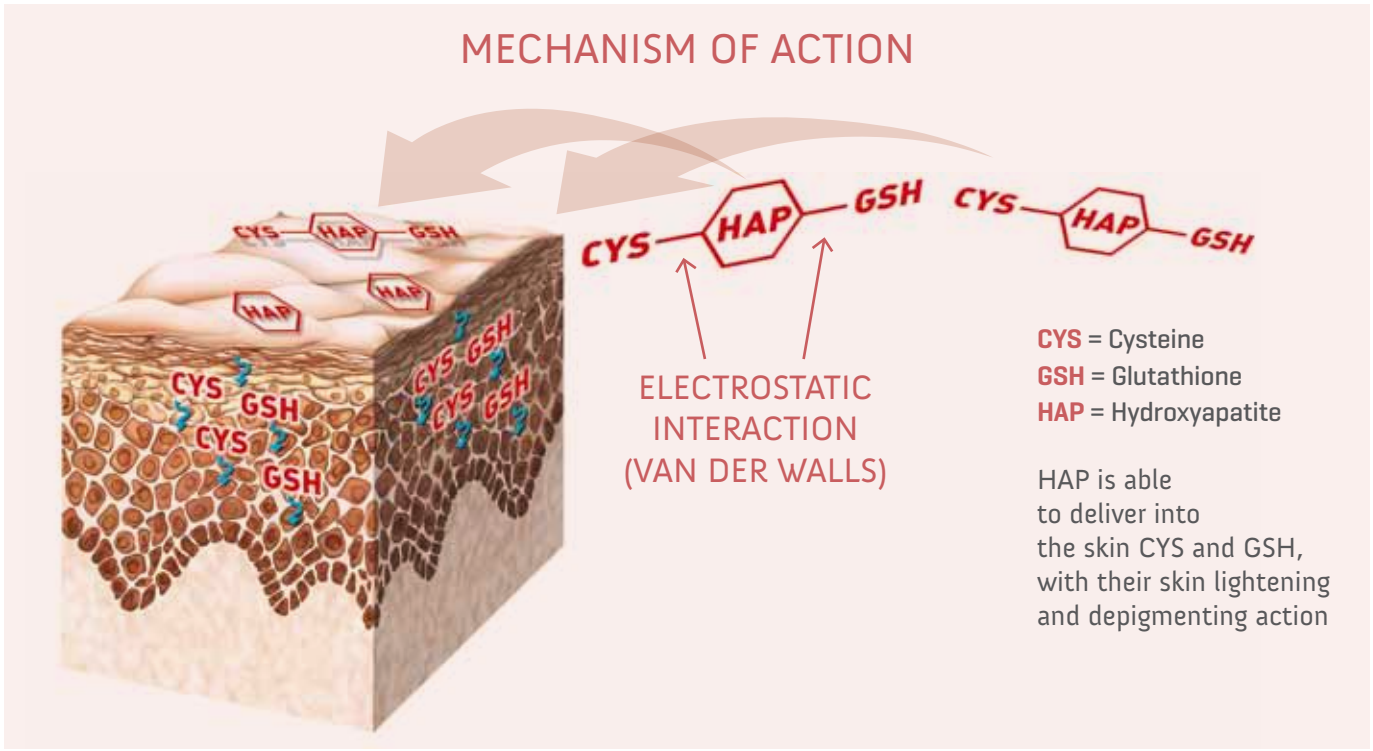


Figure 4: Action of Kalilight on the skin

>> IN VIVO TEST

LIGHTENING EFFICACY
INSTRUMENTAL EVALUATION

Instrumentation: **Chroma Meter CR 400 Minolta**
Duration of the Test: **6 weeks (2 times/day)**
# of volunteers: **24 - from 30 to 70 y.o.**

Comparison creams:

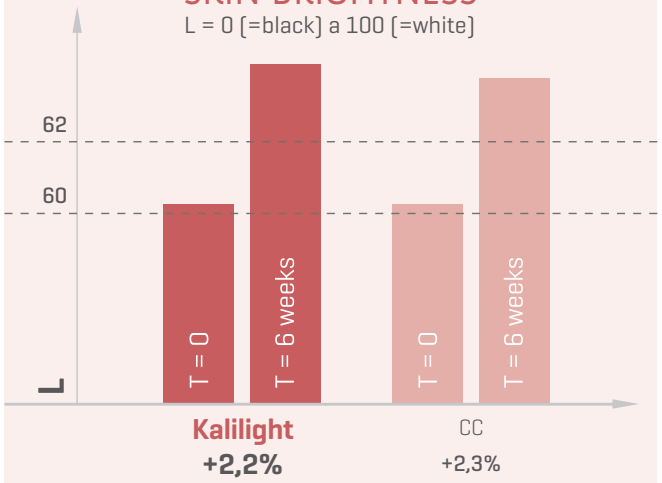
Cream with Kalilight **[Kalilight]**
Cream with lightening actives Comparison Cream **[CC]**

Measures:

Skin brightness **[L]**
Blue-yellow coloration **[b*]**
Individual Tipology Angle **[ITA°]**

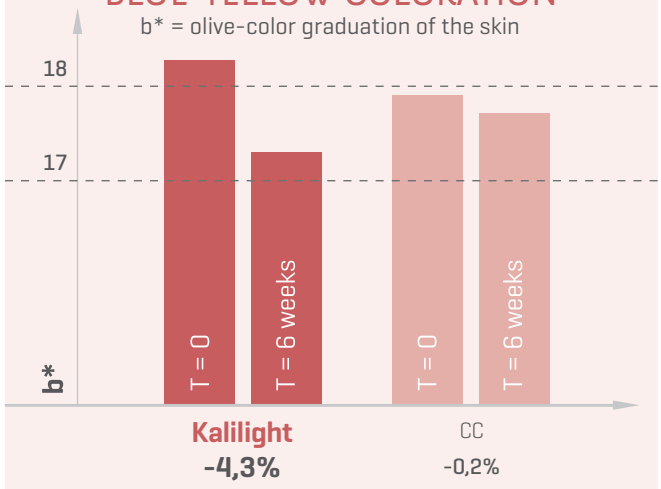
SKIN BRIGHTNESS

L = 0 (=black) a 100 (=white)



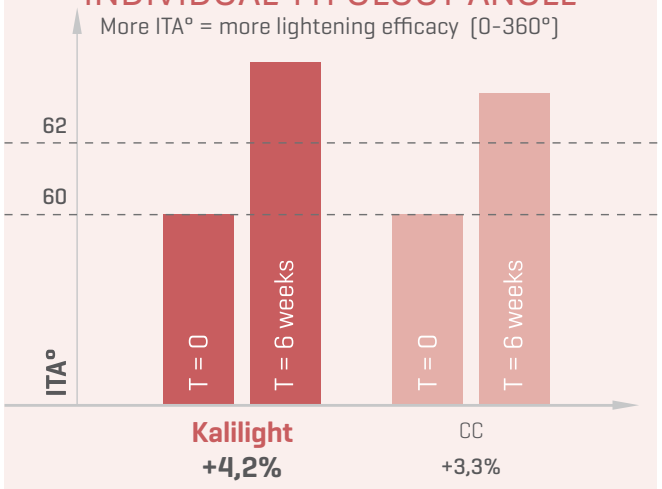
BLUE-YELLOW COLORATION

b* = olive-color graduation of the skin



INDIVIDUAL TYPOLOGY ANGLE

More ITA° = more lightening efficacy (0-360°)



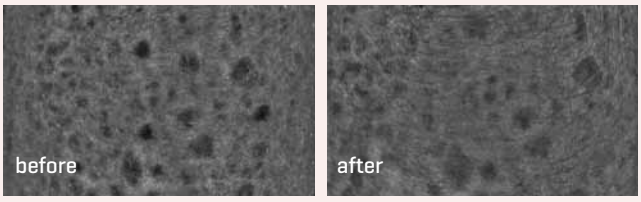
A statistically significant improvement in the melanin index [ITA°] and a statistically significant decrease of the blue yellow coloration was measured. A very important result was also achieved in the case of skin brightness.

>> IN VIVO EFFICACY

LIGHTENING EFFICACY PHOTOGRAFIC EVALUATION

An Instrumental Evaluation of the Whitening Effect on Hyper-Chromatic Skin Spots of a cosmetic emulsions has been carried out. In order to evaluate the whitening efficacy of the emulsion containing Kalilight, 24 women with hyper-chromatic skin spots applied the creams twice a day for 6 weeks. The instrumental measurements performed at the beginning and after 6 weeks of treatment showed the following results:

UV SCAN IMAGES



UV SCAN image
T = 0

UV SCAN image
T = 6 weeks

FOTOFINDER DERMOSCOPE IMAGES

Fotofinder dermoscope Ver. 2.0
T = 0

Fotofinder dermoscope Ver. 2.0
T = 6 weeks

This underlines that the treated skin spots became lighter during the 6 weeks' treatment. Visible improvements are also evidenced through UV-scan images.

>> SAFETY

Considering the composition of the active system where the main components are Hydroxyapatite, Glutathione and Cysteine, the final product may be regarded as having a low toxicological profile. Indeed, all these ingredients are naturally produced in the human organism with different physiological functions.

Cysteine

L-cysteine and L-cysteine hydrochloride are permitted food additives under Directive 95/2/EC and may also be used for nutritional purposes in processed cereal-based food and food for infants and young children (Directive 96/5/EC, Annex IV), in infant formulae and follow-on formulae (Directive 91/321/EC, Annex III) and also as nutritional substance in foods intended for particular nutritional uses (Directive 2001/15/EC). The SCF for Food evaluated the safety of L-cysteine in 1990 and decided that its use as a flour treatment agent was toxicologically acceptable provided its addition to food does not give rise to a nutritional imbalance of amino acids (SCF, 1991) (18).

>> PRODUCT SPECIFICATIONS

INCI NAME and COMPOSITION:	CAS No	EINECS / ELINCS
HYDROXYAPATITE	1306-06-5	215-145-7
GLUTATHIONE	70-18-8	231-791-2
CYSTEINE	52-90-4	200-158-2

PHYSICO - CHEMICAL ANALYSIS	METHOD	LIMITS
ASPECT	Visual	HOMOGENEUS
ODOUR	Olfactory	CHARACTERISTIC
COLOUR	Visual	WHITE
pH SOLUTION	Potentiometric	6 - 7
TOTAL MICROBE COUNT	by inclusion Ph. Eur. 7.0	< 100 UFC/g

DESCRIPTION: Micro hydroxyapatite particles activated and functionalized with complex Cysteine and Glutathione. It acts as skin lightening and depigmenting complex.
STORAGE CONDITIONS: Keep in original containers, well closed, in a cool (minimum suggested temperature 14° C. max 30° C.), dry, well ventilated and clean site.

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Kalichem Italia s.r.l.

Head Office and Production:

Via G. Pastore,1 - 25082 Brescia ITALY
Tel: +39.030.26.93.532 - Fax: +39.030.21.93.581
kalichem@kalichem.it

www.kalichem.it