

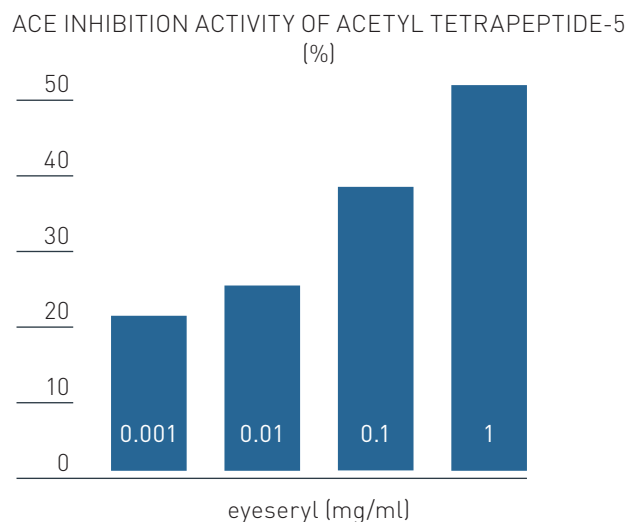
KEY

INGREDIENTS

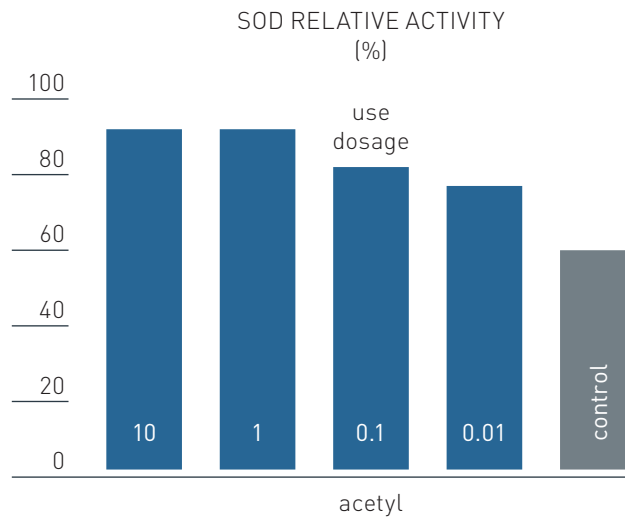
CONFIDENTIAL

ACETYL TETRA-PEPTIDE-5

As skin loses its elasticity and muscles weaken through age, loose skin can accumulate around the eyes, forming folds in the eyelids. Fat, which cushions the eyes in their sockets moves forward out of the ocular cavities and accumulates in bulging bags around the eyelids. Another reason for puffy eyes is water accumulation. Fluid may build up for several reasons, mainly because of poor lymphatic circulation and increased capillary permeability. This high technological peptide can help to drain liquid, to reduce liquid retention and to enhance skin elasticity and smoothness. It is the ideal active ingredient to fight against eye puffiness and to enhance eye contour smoothness. Acetyl tetrapeptide-5 acts at different levels and on several mechanisms. It has been suggested that one of the mechanisms involved in the formation of bags under the eyes is the bad circulation of the blood due to hypertension in this area. For this reason, a valid mechanism for an anti-puffiness compound would be the improvement of blood circulation by an anti-hypertensive effect. Angiotensin I Converting Enzyme (ACE) is involved in regulating peripheral blood pressure and can contribute to hypertension due to its cleaving enzymatic activities on physiological peptides. If ACE activity decreases, blood circulation can be improved. The ACE inhibitor activity was measured for acetyl tetrapeptide-5 and in vitro tests showed an obvious inhibitory and dose dependent effect for this peptide.



Glycation is a non-enzymatic reaction between glucose or other monosaccharides and a protein which leads to the chemical coupling through a Schiff's base between the saccharides and the protein. These compounds can interact with other molecules with irreversible reactions (oxidation, cross-linking, ...) Collagen can be subject to these irreversible reactions and collagen cross-linking is one of the main causes of the formation of eyebags. Acetyl tetrapeptide-5 is able to inhibit enzymes glycation and hence to maintain their activities. Especially SOD (Super Oxide Dismutase) can be glycated and lose its antioxidant and protective activity against superoxide radicals. Acetyl tetrapeptide-5 was tested for glycation inhibition with SOD. In vitro tests indicated an inhibitor effect against glycation and therefore a protection of SOD activity.



Vascular permeability can also play a role in puffiness and the loss of draining effect in the tissue. Acetyl tetrapeptide-5 was tested in an in vitro model vascular permeability with endothelial cell monolayers in culture inserts. Results prove that acetyl tetrapeptide-5 is able to inhibit vascular permeability in a dose-dependent manner, reaching a 50% inhibition respect to controls with 1mg/ml acetyl tetrapeptide-5. This in vitro activity would be translated in vivo in a decrease of water accumulation in eyebags, since this peptide avoids water leakage from blood vessels.

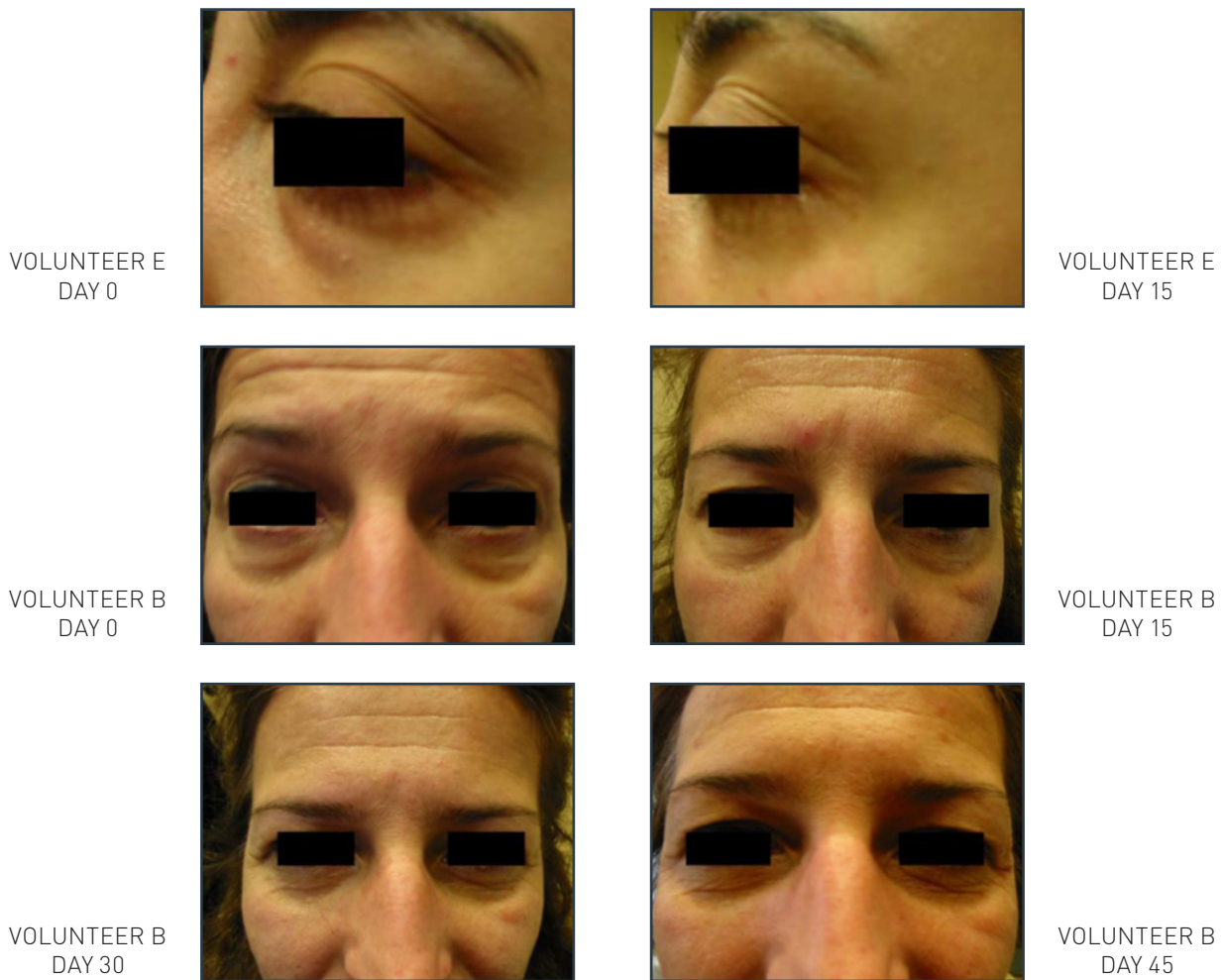
Acetyl tetrapeptide-5 was tested in vivo on a group of 20 female volunteers, aged from 18 to 65. A cream containing 0.01% peptide was applied twice a day during 60 days. Pictures were taken at different days and were measured instrumentally or visually and physically evaluated by a dermatologist. The dermatologist assigned a score to decrease in eyebag puffiness by comparison to picture at day 0 (before treatment). 1 = no reduction, 2 = slight reduction, 3 = fairly good reduction and 4 = good reduction. The dermatologist evaluations shows an increase of the effect intensity in wrinkles reduction along time while the number of grade 1 decreased dramatically and the number of grade 4 increased. Interestingly, grade 2 and grade 3 represents more than half of the volunteers at day 15 and day 30, indicating a quite fast effect which lasts. These quantifications are still confirmed by the photo taken on volunteers and appreciations.

Follow-up of eye contour of 20 volunteers according to dermatologist score ...

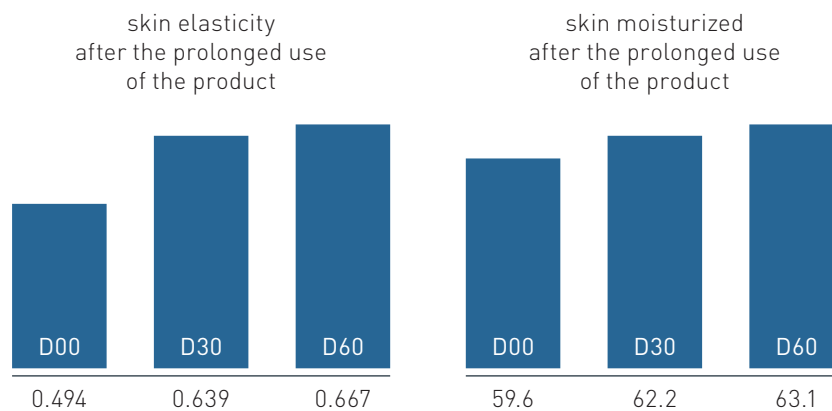
	D15	D30	D60
GRADE (1)	30%	10%	5%
GRADE (2)	25%	40%	30%
GRADE (3)	30%	25%	30%
GRADE (4)	15%	25%	35%

Puffiness under the eyes is greatly reduced, even after only D15. 70% of the volunteers had improved at D15. At the end of the test, 95% of the volunteers had improved:

- GRADE (2) = 30% showed a slight improvement
- GRADE (3) = 30% showed a fairly good improvement
- GRADE (4) = 35% showed a good improvement



The follow-up for the 20 volunteers included also characterization of skin elasticity and skin moisturization. Skin elasticity was also measured with a cutometer on the 20 volunteers. In elastometric measurement, the skin surface is aspirated from the depression induced by the machine into the aperture of the elastometer measuring probe. The depth of the skin penetration inside the probe is measured by an optic sensor. Cutaneous elasticity reflects the skin potential capacity for retraction and is expressed as the ratio: skin extensibility/residual deformation. Skin elasticity had increased by 35% after 30 days.



Skin moisturization was measured with a corneometer. It is based on the electric properties of skin surface. Indeed both electric capacity and conductance of biological tissue change according to the water content and increase with it. The results show a significant increase in skin moisturization after 60 days.

ASSOCIATION HESPERIDIN METHYL CHALCONE, DIPEPTIDE-2 & PALMITOYL TETRAPEPTIDE-3

These 3 active ingredients have been associated to make a synergy between their activities:

- Hesperidin methyl chalcone (decreases the capillary permeability)
- Dipeptide-2 enhances the lymphatic circulation
- Palmitoyl Tetrapeptide-3 improves firmness and elasticity and reduces inflammatory events

This unique combination will help to prevent and to fight against the apparition of bags under the eyes, to smooth the lines and to increase skin firmness and tone. 20 volunteers using this active association daily were evaluated by 3D morphometric studies and asked for auto-evaluation.

before
treatment



after
treatment

The morphometric study gives significant results after day 28 and 56: respectively, the bags thickness diminished in average to -0.08 and -0.20 mm with maximum values of -0.40 at day 28 and -0.69 at day 56. 65% and 70% of the volunteers experienced this decrease of bags under the eyes. Concerning auto-evaluation, 62% found that the eye contour was smoother, 52% that the bags diminished and 52% felt a decongesting effect.

CHONDROITIN SULFATE

This oligosaccharide from marine origin is obtained from depolymerization of glycosaminoglycans (GAG), one of the major component of extracellular matrix. It can stimulate the cell neosynthesis and glycosaminoglycan synthesis, helping in regeneration and restructuration of skin. Due to its high capacity of retaining water, it is also an excellent moisturizing agent.

EUCALYPTUS ESSENTIAL OIL

Eucalyptus essential oil is well-known for its sanitizing properties, especially for antiseptic properties. In HPR Mask, it will bring purifying properties in synergy with kaolin. Physically and biologically, the skin is purified.

GLYCOLIC ACID

Glycolic Acid is one of the famous Alpha-Hydroxy Acids (AHA). AHA or fruits acids were primarily extracted from, which gives their name and concern a family of molecules containing an acid function very close to an hydroxyl group. They have been used since the Antiquity and some specialists suspect that even Cleopatra used this type of active ingredients to improve her complexion and her beauty. AHA can enhance the natural process of "exuviation" or exfoliating process to renew skin cells on the surface. They are able to cleave intercellular bonding within the stratum corneum and to release fragments of dead cells and other impurities.

HYDROLYZED HAZELNUT PEPTIDES

This molecule is a peptide extract of hazelnuts with a low molecular weight (greater bioavailability). Thanks to its wealth of peptides, amino acids and sugars (glucose, galactose), it stimulates the growth of the two main specialist cells found in the skin: keratinocytes and fibroblasts, whilst enhancing their respective metabolism. It also triggers cell differentiation process of epidermis, which is essential for remodeling the skin and restoring its barrier function. PROPERTIES: stimulation of cell renewal, cell metabolism, fibroblasts-collagen interactions, cell cohesion, and cell differentiation.

Evaluation of the effect of peptide extract from hazelnuts on the growth of normal human keratinocytes and fibroblasts.

Effect of Peptide extract from hazelnuts on the growth of human keratinocytes:

	Control	EGF (epidermal growth factor) 0.01%	Peptide extract from hazelnut 0.01%
Activity in %	100	167	233

Effect of Peptide extract from hazelnuts on the growth of human fibroblasts:

	Control	FCS (fetal calf serum) 10%	Peptide extract from hazelnut 0.01%
Activity in %	100	154	180

Evaluation of the effect of Peptide extract from hazelnuts on extracellular collagen neo-synthesis in human fibroblast cultures:

	Control	Vitamin C 100µM	Peptide extract from hazelnut 0.01%
Activity in %	100	139	170

HYALURONIC ACID *

Hyaluronic acid is a linear biopolymer, member of the class of amino-sugar containing polysaccharides, known as glycosaminoglycans (GAGs). Hyaluronic acid occurs naturally in various body tissues such as skin and cartilage. It is suitable for biomedical applications which target these tissues. Hyaluronic acid is now commonly used as an ingredient in cosmetics due to its moisturizing properties. Recently, the latest research have reduced the molecular weight of the hyaluronic acid so that it can be absorbed internally, using a bacterial fermentation that secrete filaments of hyaluronic acid. The effects are cellular regeneration, skin cells mediation, hyaluronan production, and cell reinforcement.

High molecular weight hyaluronic acid solutions form non occlusive hydrated visco-elastic films when applied on the skin surface, keeping possible water perspiration. Those films hold water in the same way that hyaluronic acid holds water in the intercellular matrix of dermal connective tissues. They also protect the skin against outer aggressions that could break the hydro-lipidic barrier and lead to the desquamation of the Stratum Corneum (Gallot, "les protéoglycanes", actifs & additifs en cosmétologie, Lavoisier TEC & DOC, p. 110-124, 1992).

It has been demonstrated by studying several moisturizers that the most efficient polymer for skin hydration should have the following characteristics:

- A high molecular weight.
- A high affinity towards water.
- A heterogenous structure that allows the polymer to form a flexible film on the skin surface.

Thus hyaluronic acid, which exhibits all those characteristics, appears as an ideal moisturizer.

Improvement Rate Of High Molecular Weight Hyaluronic Acid VS Placebo
(journal of new remedies & clinics, vol. 50, # 5: 90 (548)-102 (560), 2001):

FACE	Product	NoS w.s.	Dol m.imp.	Dol imp.	Dol unch.	Dol exa.	Dol m.exa.	Imp. RATE	SoD
RMU	HA	3	0	3	0	0	0	100.0%	
	placebo	4	2	0	2	0	0	50.0%	
Dryness	HA	11	5	5	1	0	0	90.9%	p<0.01
	placebo	11	1	3	7	0	0	36.4%	
Flush	HA	9	3	5	1	0	0	88.9%	
	placebo	6	0	3	3	1	0	50.0%	
HMU	HA	6	2	2	2	0	0	66.7%	
	placebo	4	0	1	3	0	0	25.0%	

NoS = number of subjects (with symptoms)

Dol = degree of improvement (markedly improved, improved, unchanged, exacerbated & markedly exacerbated)

SoD = significance of difference

RMU = rash caused by make-up

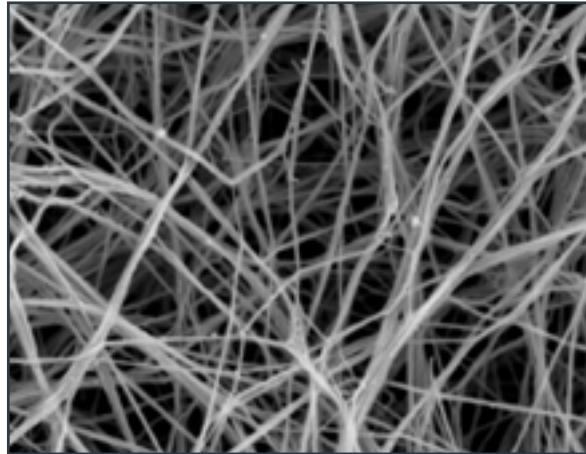
HMU = hardness to apply make-up

As we can see, high molecular weight hyaluronic acid induces an improvement in 66-100% of the subjects' symptoms.

(*) Biotechnologically sourced high molecular weight sodium hyaluronate reproduces the natural hydration characteristics of hyaluronic acid. Sodium hyluronate only derives from natural products and from an ecological process: a plant substrate, providing bacteria with the ingredients they need (glucose, nitrogen, and minerals), and the use of bioethanol.

HYDROLYZED MARINE COLLAGEN

Collagen, of course, occupies a central place in the ageing process. If all the different forms of collagen are taken together (19 forms have been identified), then it is the most abundant protein in mammals (25% of the total protein). It is also the main constituent of the extra-cellular matrix, and has a specific triple helix, rope-like structure. Protein fibrils are organised to form bundles that are arranged at right angles to each other and held in place by inter-fibre cross linkages: this produces a network structure and confers mechanical resistance on the skin. The most abundant collagen is type 1 collagen.



Collagen is the substance that protects and supports the soft tissues of the body and connects them to our skeleton. In other words, it's the material and the glue that holds our body. The collagen "colla and genmen" means "produce glue". The collagen is the glue of the body. It gives to different organs and tissues their strength and elastic properties. 25% of protein's weight in the human body is composed of collagen. 75% of our skin is made of collagen and about 30% of our entire body is made up of collagen. Collagen is part of the normal composition of natural tendons, ligaments, joints, muscles, hair, skin and vital organs. When there is a lack of collagen in the body, everything is affected. Hydrolyzed collagen has showed its activity at high doses in many clinical trials (ITE letters on batteries, new technologies & medicine, vol. 7, # 4, 2006).

HYDROLYZED MARINE COLLAGEN IMPROVEMENT RATE

Symptom	NoS with symptom	DoI improved	DoI unchanged	DoI exacerbated	Improvement RATE
FD	25	23	2	0	92.0%
HMU	22	21	1	0	95.5%
RMU	5	5	0	0	100.0%
Flush	13	10	3	0	76.9%
Pimple	15	13	2	0	86.7%
Wrinkle	25	15	9	1	60.0%
Speckle	25	18	7	0	72.0%
UDC	20	19	1	0	95.0%
Dropsy	15	10	4	1	66.7%
BI	21	15	6	0	71.4%
Dryness	25	20	4	1	80.0%
Flush	11	9	2	0	81.8%
Exfoliation	19	14	4	1	73.7%
Vesicle	6	5	0	1	83.3%
TOTAL	247	197	45	5	79.8%

NoS = number of subjects
 DoI = degree of improvement
 FD = face dryness
 HMU = hardness to apply make-up
 RMU = rash caused by make-up
 UDC = undereye dark circle
 BI = body itchiness

As we can see, hydrolyzed collagen induces an improvement in 60-100% of the subjects' symptoms (ITE letters on batteries, new technologies & medicine, vol. 7, # 4, 2006). Hydrolyzed Collagen has a technology that rebuilds the skin matrix at low doses. This product is manufactured with the highest standards of biotechnology and answers to all health requirements, all over the world.

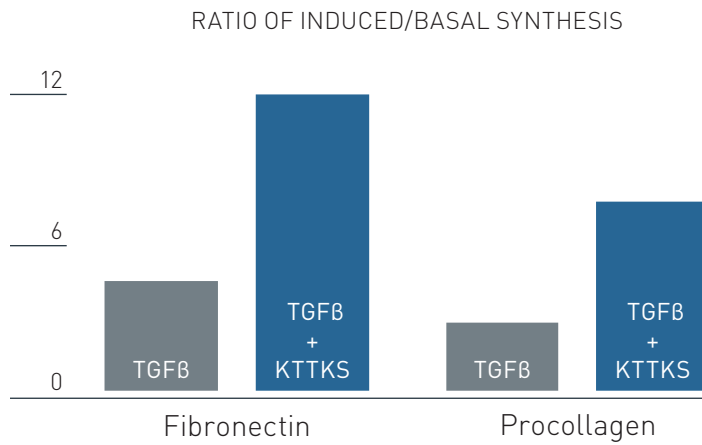
SUMMARY OF THE IN VITRO DATA

obtained by Katayama for Lys-Thr-Thr-Lys-Ser (KTTKS)

The studies were carried out using human lung fibroblasts (HFL-1) cultured in DMEM culture medium + 10% FCS (foetal calf serum). The stimulation of collagen synthesis was monitored from the incorporation of tritiated proline, electrophoresis and fluorometry. Many of the peptides corresponding to sequences found within the 197-223 fragment of procollagen 1 have been synthesised and tested in the culture medium at sub-confluence. The 20-amino acid peptide (197-216) results in a stimulation of the synthesis of the extra-cellular matrix (ECM) by about 400%, which is characterised by the presence of α 1-, α 2-procollagen and fibronectin. Pentapeptide KTTKS (residues 212-216) has 80% of this activity, i.e. at a concentration of 50 μ M it produces a 320% increase. Other peptides with a similar sequence and shorter peptides display little activity.

The KTTKS sequence therefore seems to be highly specific for interaction with fibroblasts. The peptide is also able to increase the cell's reaction to TGF β (transforming growth factor), which has been shown to stimulate the biosynthesis of the extracellular mesh.

The amount of fibronectin synthesised in the presence of TGF β and of KTTKS is three times greater than in the absence of this peptide and then synthesis of procollagen is potentiated by a factor of 4.4.



ex-vivo test: neosynthesis of collagen by a human skin explant

HYDROLYZED SESAME PROTEIN

Original, patented sesame-based molecular combination. This tightening agent instantly and visibly smoothes the skin. Rejuvenation, firmness, moisture-replenishment and comfort guaranteed (proven in vivo effect) for anti-ageing formulas. PROPERTIES: erases wrinkles thanks to its "tightening" effect (flash effect), moisturizing effect persists after 4 hours as well as skin comfort and softness on application.

IMMEDIATE AND VISIBLE ANTI-WRINKLES EFFECT

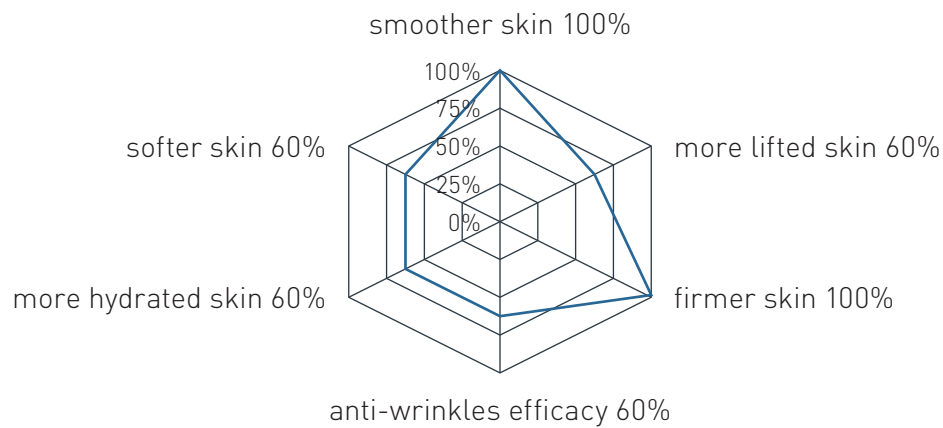


(before application)



(5 minutes after application 10% hydrolyzed sesame protein diluted in water)

Sensorial effect felt by the women 5 and 30 minutes after hydrolyzed sesame protein application (clinical test). Comfortable on application, hydrolyzed sesame protein leaves the skin soft and silky. Skin tightening without any unpleasant feel.



The active protein, extracted from hazelnut, can fight against two key processes of cells ageing: differentiation and proliferation. These two processes slow down natural cell proliferation and hence prevent from cell renewal and cell regeneration. On the other side, ageing prevents from biotransformation of skin cells to other cells types, essential for the structure of the skin. Hydrolyzed Hazelnut Protein is able to play key roles in these mechanisms. For example, in vitro tests indicate that it can increase markedly keratinocytes and fibroblasts. It can promote collagen neo-synthesis in human fibroblasts culture. It is able to promote cell differentiation, which can be proved for example by increasing the transglutaminase keratinocyte TGK1 enzyme. This enzyme is an important market of the cell differentiation. Activation of this enzyme improves the barrier function of the skin and ensures preservation of its integrity. It then becomes less susceptible to environmental aggression and skin hydration is improved.

KAOLIN

This mineral powder is insoluble and has excellent absorbent properties. As it is under a microparticle form, its specific surface is very high and leads to great absorption capacities. It can draw out impurities, dirt and toxins and remove excess of oil in a gentle way. It is suitable for all skin types. Even the skin feels its effects and feels purified.

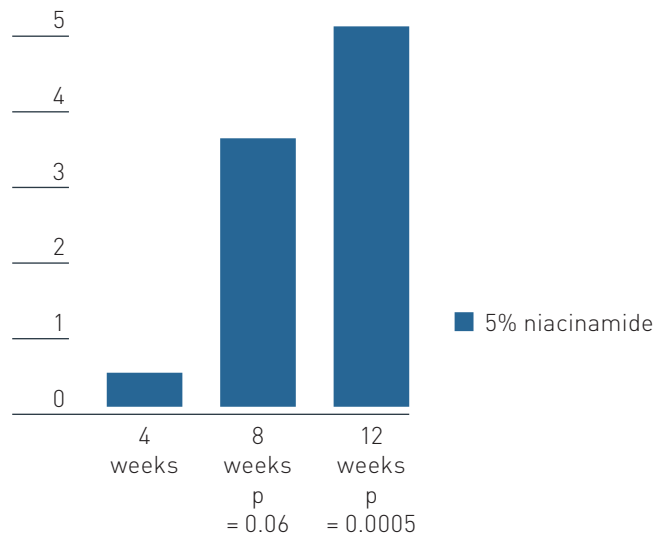
MACADAMIA OIL

For thousands of years, the macadamia nut has been eaten by the Australian Aborigines as a 'bush-food' for its high nutritional and energy value and health promoting benefits for radiant skin and shiny hair. Obtained by cold pressure without solvent, it contains more than 80% of unsaturated fatty acids, leading for excellent anti-oxidative stability and high interest for skin needs. It can bring palmitoleic acid, which rate decreases with age. Its composition is particularly adapted to balance the lipid content of the skin.

NIACINAMIDE

In multiple chronic clinical studies, topical niacinamide [vitamin 83] has been observed to be well tolerated by skin and to provide a broad array of improvements in the appearance of ageing facial skin. RESULTS: Analyses of the data revealed a variety of significant skin appearance improvement effects for topical niacinamide (reductions in fine lines and wrinkles, hyperpigmented spots, red blotchiness, and skin sallowness [yellowing]). In addition, elasticity is improved. Topical niacinamide reduces fine lines/wrinkling in facial skin. Subjects were female caucasians (n = 50) who applied placebo control vs. topical niacinamide (twelve weeks, split face, left/right randomized clinical trial).

REDUCTION IN TOTAL WRINKLE LENGTH VS. PLACEBO CONTROL
%

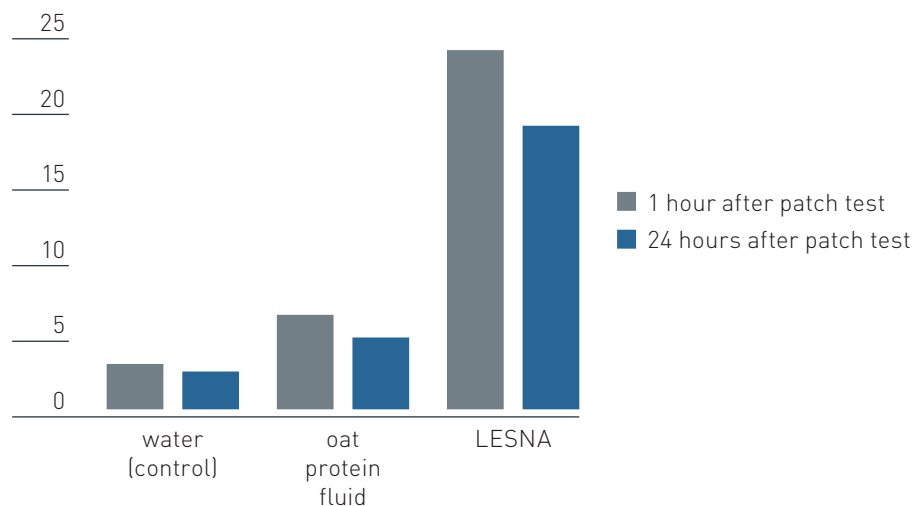


OAT PROTEIN DERIVATIVES

Anionic surfactant derived from amino acids characteristic of oats. Our oat protein fluid is ideal for cleansing with a fine foaming texture. A surfactant whose creamy, soft texture respects the skin barrier of sensitive skin. PROPERTIES: abundant foam texture, gentle cleanser (respects the skin barrier) and non delipidating.

WATER LOSS

Instant foaming cleanser counteracts skin de-lipidation when washing and promotes re-lipidation in vivo study. The results show that the instant foaming cleanser is softer than classic anionic cleansers.



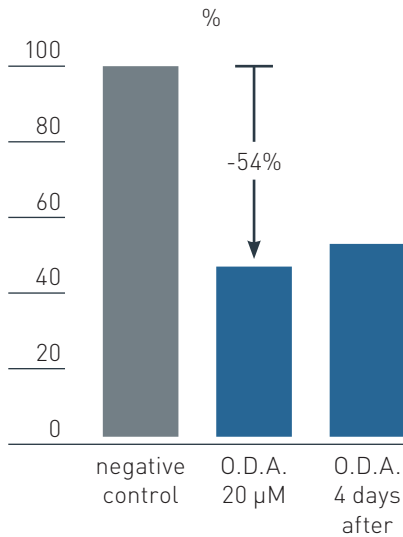
OCTADECENEDIOIC ACID

Octadecenedioic acid is able to brighten the skin by reducing melanin synthesis. It helps inhibit the entire metabolic pathway of melanin synthesis from the nucleus of the melanocyte.

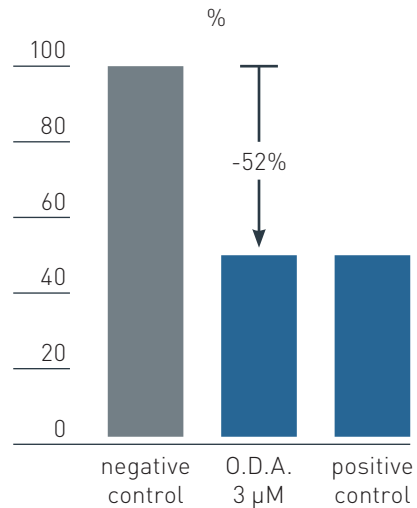
IN VITRO TEST

Activity on tyrosinase mRNA, tyrosinase & melanin level. RESULT: by reducing melanin level, the skin is brightened and looks more radiant.

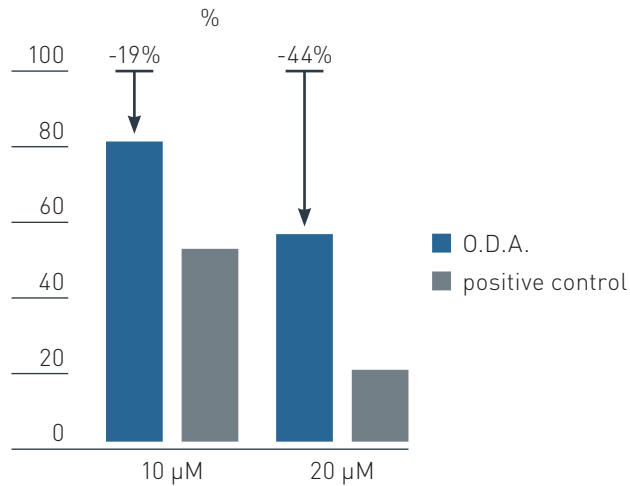
TYROSINASE MRNA QUANTITY VS. CONTROL



TYROSINASE QUANTITY VS. CONTROL



PIGMENTATION AS A FUNCTION OF O.D.A. CONCENTRATION

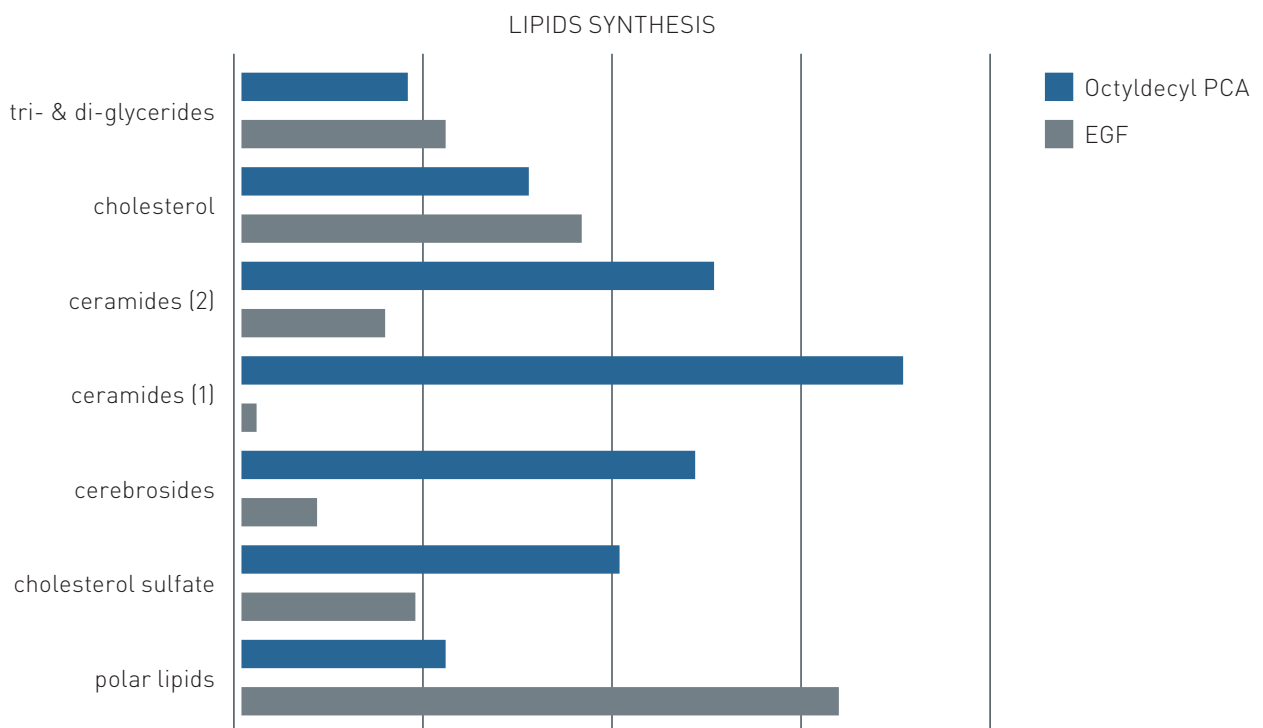


OCTYLDECYL PCA (PYRROLIDON CARBOXYLIC ACID)

Providing exogenous lipids represents the first step of the treatment but it is not sufficient for dry skins. It is essential to repair and to reinforce the cutaneous barrier by stimulating endogenous lipids synthesis. Especially ceramides are the main components of the intercellular cement in the stratum corneum and play a key role in cutaneous barrier integrity. We select the physioester Octyldecyl PCA for its particular structure which confers skin restructuring properties through biomimetism. Its double lipid chain is very similar to those found in cell membrane lipids. Due to its particular amphiphilic structure and chemical composition, Octyldecyl PCA presents an excellent affinity for stratum corneum. It is also easily integrated in formulation due to its high capacity of association and self organization. In addition to this, it gives a very soft and non-greasy touch.

EX VIVO TEST

Octyldecyl PCA was tested ex vivo for stimulation of lipids synthesis on human skin explants and was compared to EGF (epidermal growth factor). The results are presented as stimulation ratio with paraffin oil as standard and indicate that Octyldecyl PCA can stimulate specific lipids synthesis in a high ratio: ceramides 1 and 2, essential lipids for skin cells, and cholesterol and derivative, essential for cell membrane structure and integrity. Octyldecyl PCA helps strongly to reinforce the cutaneous barrier.



Octyldecyl PCA stimulation compared to EGF stimulation on different lipids synthesis
(ex vivo tests on human skin implants/% are calculated as ratio)

OLEYL ERUCATE

Oleyl Erucate from Jojoba Oil different from other common plant oils in that it is composed almost completely (97%) of wax esters of monounsaturated, straight-chain acids and alcohols with high molecular weights (carbon chain lengths from 36 to 46). This makes it more similar to sebum and whale oil than to traditional vegetable oils.

PALMITOYL PENTAPEPTIDE-4

Palmitoyl Pentapeptide-4 is an amphiphilic analogue of a collagen I peptide sequence.

Why have we chosen the specific sequence LYS-THR-THR-LYS-SER? This pentapeptide, which is a fragment of the C-terminal portion of collagen I, has been studied in detail by Katayama et al. When this peptide is added to cultured human fibroblasts it enhances the synthesis of collagen I, collagen III and fibronectin (Ayock et al., 1986; Katayama et al., 1991). It repairs the dermal matrix, stimulates fibroblast growth and increases the synthesis of collagen I and IV, and other structural molecules of the epidermis.

How can the synthesis capacity of the fibroblast be reinvigorated? Apart from the steroid hormones and their derivatives, which are not suitable for cosmetic use, the "feed-back" peptides, which are fragments of the matrix protein, provide an appropriate approach. They are selective natural messengers are immediately recognised by the cell.

The increased lipophilic character of this substance enhances its affinity towards the skin and also has the advantage of enhancing the chemical stability of the peptide and making it more bioavailable (Lintner & Peschard, 1999). Similar vectorisations have already been shown to work for other peptides (biopeptide CL, biopeptide EL, sederma patent, 1990). In vitro and ex vivo studies carried out using cultured fibroblasts or explants have been designed to validate the reconstruction properties of the extracellular matrix and to demonstrate the biological activity of the peptide in models corresponding to cosmetic interests. A major in vivo study completes these investigations and has demonstrated the efficacy of palmitoyl pentapeptide-4 in repairing wrinkles. Conclusions are that palmitoyl pentapeptide-4 and vitamin C stimulate the synthesis of the extra-cellular matrix in vitro and ex vivo to very different extents: collagen I = +117% VS +42% for vitamin C; collagen IV = +327% VS +229% for vitamin C & palmitoyl pentapeptide-4 also stimulates the production of glycosaminoglycans: +267%.

The main support structures of the dermis and epidermis are therefore stimulated in a physiological manner. Palmitoyl pentapeptide-4 can correct the ageing process and promote the reappearance of the characteristics of a younger skin. The previous findings have been confirmed by a 6-month, blind, placebo-controlled, in vivo study. A 3% Palmitoyl Pentapeptide-4 cream progressively effaces the wrinkles. After 6 months the effects were as follows:

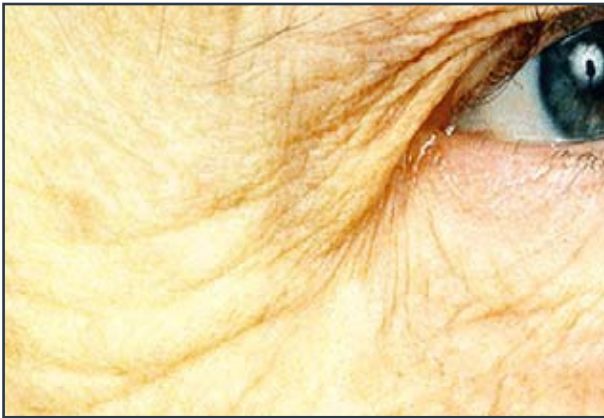
- The surface area of the deep wrinkles decreases by -68%, and in some cases was reduced by as much as 90%
- The density of the main furrows by -47%
- The area of the moderate wrinkles by -51%
- The mean area of the main wrinkle by -22%

Tested blind versus a commercial product containing 5% vitamin C, palmitoyl pentapeptide-4 confirmed that it was more effective and is also stable and is well tolerated. Palmitoyl pentapeptide-4 was very well tolerated. With the 3% palmitoyl pentapeptide-4 cream, 80% of the volunteers could see a visible reduction in their wrinkles. These observations have been confirmed by the dermatologist's clinical examination, which revealed a clear improvement on the palmitoyl pentapeptide-4 side. They are shown in the macrophotographs taken during the examinations.

PROFILOMETRIC INVESTIGATION OF THE PARAMETERS

- Highly significant changes at T2 months, T4 months and T6 months versus T0 for the 3% Palmitoyl Pentapeptide-4 cream versus the placebo.
- Significant changes after T6 months versus T0 for the 3% Palmitoyl Pentapeptide-4 cream versus the cream containing 5% Vitamin C.

These effects (recognised equally by both the panel volunteers and the dermatologist) were also visible, as can be seen from the photographs below:



T0



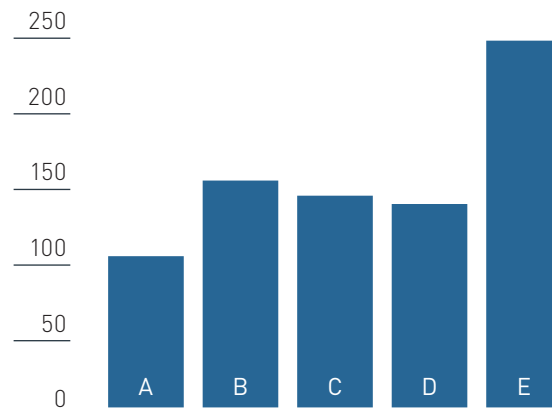
T6 months

IN VITRO TESTS

Skin biopsies obtained from an abdominal plasty in a 43-year old subject were maintained under physiological conditions for 48 hours. The biopsies were exposed to various concentrations of Palmitoyl Pentapeptide-4 or to vitamin C (reference substance) or TGFβ (natural growth factor). Biopsies in control culture medium containing no Palmitoyl Pentapeptide-4 were processed in parallel.

TGFβ, the positive control, increased the synthesis of collagen by 33%. At both concentrations tested, Palmitoyl Pentapeptide-4 produced major stimulation of the synthesis of collagen, with a maximum response of 117% at the 4% concentration, which was greater than that of either TGFβ or vitamin C.

COLLAGEN SYNTHESIS



- A = Control
- B = Vitamin C
- C = TGFβ
- D = Palmitoyl Pentapeptide-4, 2%
- E = Palmitoyl Pentapeptide-4, 4%

IN VIVO TEST

The test lasted 6 months and involved a panel of 35 female volunteers between 34 and 72 years of age (group mean: 58 years). The anti-wrinkle effect of a cream containing 3% Palmitoyl Pentapeptide-4 was investigated in several ways:

- Profilometry and image analysis
- Photography
- Clinical examination by a dermatologist
- Self-evaluation by the subjects themselves.

The study was carried out under double-blind conditions versus a placebo and a reference substance.

RESULTS

A highly significant reduction in the deep and moderate wrinkles was obtained on the side treated with 3% Palmitoyl Pentapeptide-4. This reduction increased with time and a difference developed from the placebo, which never produced a significant effect. The surface area occupied by deep wrinkles was reduced by a mean of 68% over 6 months. The surface area occupied by moderate wrinkles was reduced by a mean of 51% over 6 months. The volume of the wrinkle decreased with time, successively being reduced by 7% (2 months), 21% (4 months) and 24% (6 months). The placebo continued to produce no effect. The effect of Palmitoyl Pentapeptide-4 intensified with time as the statistical analysis demonstrates. The changes in the "roughness" parameter takes into account the effects obtained on the reduction of the changes in skin relief, corresponding to a general smoothing of the skin. Whereas the placebo did not reduce the roughness of the skin (very slight increase of no significance), Palmitoyl Pentapeptide-4 revealed an effect after 2 months (-10%) which intensified with time to reach -16% after 6 months. The mean depth of the main wrinkle decreased throughout the treatment (up to 21% after 6 months). This study demonstrates the long-term anti-wrinkle activity of Palmitoyl Pentapeptide-4. The parameters used to compare the Palmitoyl Pentapeptide-4 cream with the placebo cream were significant throughout the treatment. These differences increased with time, which indicates that the physiological activity of the peptide in the skin was progressive. The clinical study carried out under blind conditions by a dermatologist, confirmed by the volunteers' self evaluation, demonstrated the greater efficacy on the 3% Palmitoyl Pentapeptide-4-treated side than that treated with the placebo or the commercial cream containing 5% vitamin C.

PANTHENOL

D-Panthenol, especially dedicated to sophisticated cosmetics, improve the appearance of skin. It is the provitamin of panthotenic acid (also known as vitamin B5) and the precursor of coenzyme A. It plays a key role in lipid metabolism, especially in cell membrane repair. It is involved in repairing the cutaneous barrier, improving hydration, decreasing indirectly inflammation reactions. As the barrier function of the skin is restored, the skin is more able to protect against external aggressions. D-panthenol can also stimulate cell growth, especially fibroblasts and hence in repair mechanisms. Due to its hygroscopic nature, it improves moisturisation on skin.

PHOSPHATIDYL CHOLINE

Phosphatidyl choline is one of the major components of the physiological membrane lipids, also called "essential phospholipids". It is composed of choline and omega 6 lipids (mainly linoleic acid). As this type of lipids cannot be synthesised by our body, they needed to be brought by food and/or also by direct application on skins. It is also involved in intracellular messengers and signaling mechanisms, essential for cell growth. They are needed for cell membrane construction and structuration. We choose this type of phosphatidyl choline, extracted from soybean, due to its particular and biomimetic lipidic composition. Its high properties of self-organisation helps to structure and maintain cell membrane.

POLYACRYLIC ACID

Polyacrylic Acid is an intelligent polymer which is able to retain a great quantity of water and to release it when needed, due to osmosis mechanisms: the water goes where it misses. A comparative study shows an increase of 44% on moisture between formulation with and without Polyacrylic Acid. In addition to this way of hydration, the effects are visible on the rugosity and microrelief of skin. With its great capacity of retaining water and releasing it, Polyacrylic Acid is ideal to moisturize the "thirsty" skin cells.

RASPBERRY SEED OIL

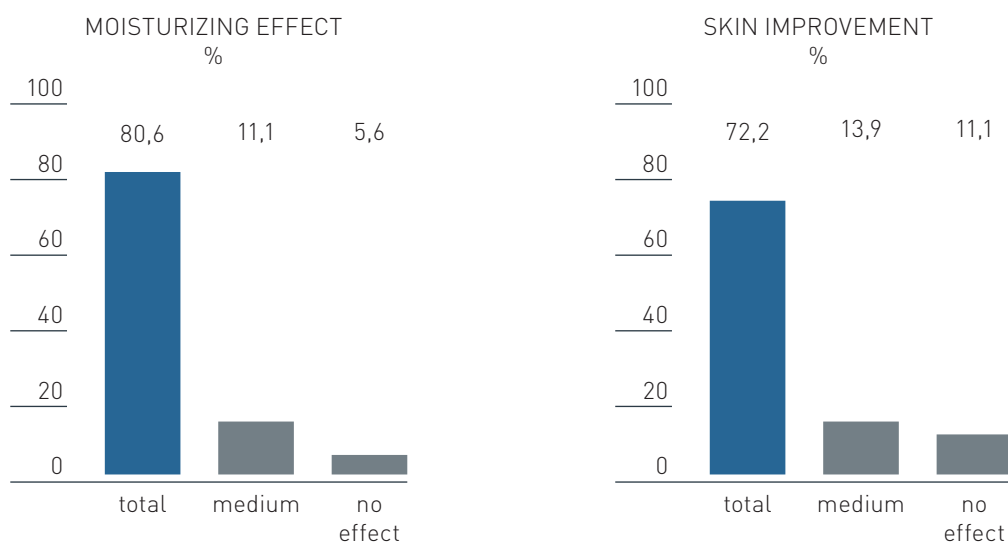
Raspberry Seed Oil (*Rubus idaeus*) is rich in Omega 6 and Omega 3 polyunsaturated fatty acids, known for their moisturizing, protecting and anti-ageing properties. This type of oil increases indeed the restructuration of cells and has a strong power to moisturize skins. Alpha linoleic acid (Omega 3) is a precursor of other major Omega 3, especially EPA (EicosaPentaenoic Acid) and DHA (DocosaHexanoic Acid). Linoleic Acid is the precursor of the Omega 6 family. In order to be metabolized by the body, it has to be converted in gamma linolenic acid. Omega 3 and 6 fatty acids play an important role in the restructuration of the skin cells. We select high quality and special grade of raspberry oil, boosted with natural tocopherols from tea extract (*Camellia sinensis* extract) to warranty the lipidic profiles and their integrity. Natural delta-tocopherols are also added to the formulation for their protective activity against lipid oxidation, their soothing properties and also for skin protection.

SAFFLOWER OIL/PALM OIL AMINOPROPANEDIOL ESTERS (CERAMIDS ANALOGS)

Ceramides are undeniably the masters of cell cohesion. Thanks to their specific structure they form with certain other epidermal lipids, the multilamellar lipid matrix found in the superficial layers of the epidermis, the role of which is to maintain skin moisturisation and provide optimal protection against external attack. However, this molecular arrangement would not be as effective as it is, if it didn't have an essential and fundamental fatty acid: linoleic acid. Currently at the top of the list of food additives for its role in regulating our body cholesterol levels, safflower oil/palm oil aminopropanediol esters are more stable than linoleic acid but guarantee the function of the stratum corneum bilayers. It presents a real restructuring effect on skin cells layers.

SESAMUM INDICUM OIL, TRITICUM VULGARE GERM OIL & SELECTED VEGETAL OILS

This composition of sesam oil and wheat germ oil is rich in essential fatty acids (EFA) combining the benefits of natural botanic oils and vitamin E for a perfect protection from dehydration and inflammation mechanisms. Thanks to its balanced combination, it fills in cutaneous lipid deficiencies, a vital key to protect, moisturize and smooth. These two mixed oils were tested in a lotion on 72 women with a daily use. After one week, more than 80% found a real moisturizing effect and more than 70% noticed skin improvement. We add macadamia oil for its composition of fatty acids and natural tocopherols for their anti-oxidant and protective properties for the skin.



VEGETAL EXTRACTS LAMINARIA, CUCUMBER & GINKGO BILOBA

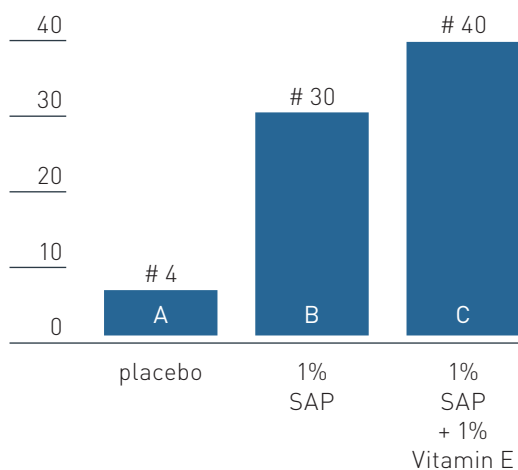
Thanks to its high content of mineral salts, laminaria is used for remineralizing the epidermis. Amino-acids, essential for physiological reactions, enable skin cells renewal and protect the hydrolipidic film. Cucumber extract can boost these actions due to its content of mineral salts, amino-acids and antioxidant components. It has been traditionally used to hydrate and sooth the skin. Ginkgo biloba, symbol of longevity has been used since Antiquity in China for its therapeutic properties. Its high contents of antioxidant such as flavoloids is interesting for every anti-ageing treatment, especially for skin. It also brings essential nutriments for cells.

VITAMIN C (SODIUM ASCORBYL PHOSPHATE)

Vitamin C (ascorbic acid) is one of the most widely used antioxidants for protecting the skin. Unfortunately, it is easily depleted when the skin is exposed to the sun, and by external stresses such as pollution and smoking. Maintaining adequate levels of vitamin C is, therefore, important to help protect the skin against UV-induced free radical damage that is related to skin ageing. To provide the maximum benefit from vitamin C, it is recommended that a stable form of vitamin C be used in personal care preparations. One such stable form of vitamin C, known as sodium ascorbyl phosphate, maximizes the protective properties of vitamin C by retaining its effectiveness over time. Sodium ascorbyl phosphate, alone or together with vitamin E, can provide an effective antioxidant combination that reduces the formation of free radicals and stimulates collagen synthesis (which slows down with ageing). Additionally, sodium ascorbyl phosphate can help to improve the appearance of skin as it can lessen the appearance of photo-damage and age spots.

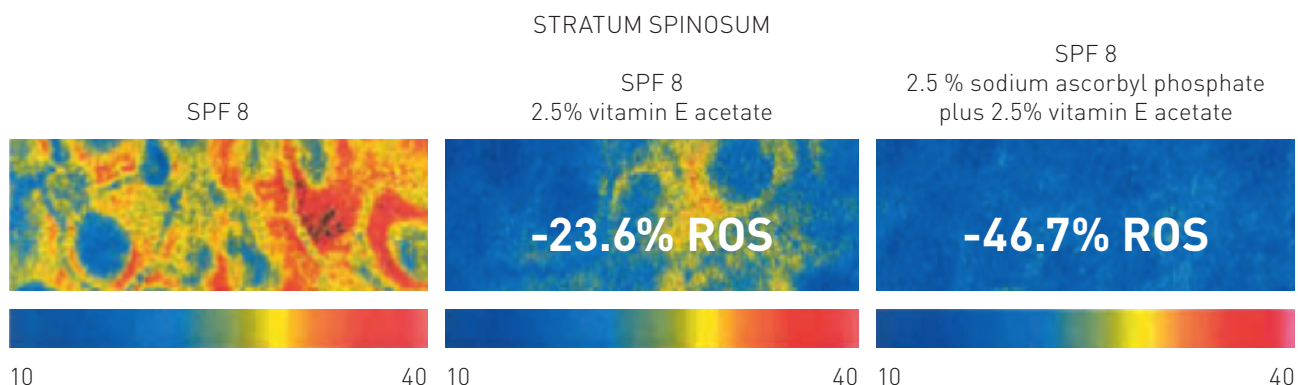
IN VIVO TEST

In a study on 20 subjects were selected sites on the volar forearm were pretreated by topical application of three different formulations containing 1% Sodium Ascorbyl Phosphate (SAP), 1% Sodium Ascorbyl Phosphate plus 1% Vitamin E Acetate or the corresponding placebo. After 7 days of application (twice per day), the skin was irradiated with a UVA dose of 10J/cm² and squalene peroxide found on the upper layers of the treated skin was identified by HPLC measurements.



inhibition of peroxidation in % (# p < 0.05 versus placebo)

The following pictures were generated using a two-photon fluorescence imaging method. Human breast tissue from plastic surgery was pretreated with different formulations prior to UV irradiation.



(RED = maximum number of ROS generated, BLUE = minimum number of ROS generated; ROS = singlet oxygen, hydrogen peroxide, superoxide and nitric oxide etc.)

... *powered by* **E S P A**

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